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ON THE PHYSIOLOGY OF TUBER FORMATION IN POTATOES

IV. INFLUENCE OF VITAMIN C ON TUBER AND SPROUT FORMATION OF POTATOES ASEPTICALLY GROWN IN THE DARK

by D. A. VAN SCHREVEN

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INTRODUCTION

The rôle of vitamin C in plants had recently been discussed by Schopfer[12]. He comes to the conclusion that it is doubtful whether ascorbic acid functions as a true growth factor. The rôle of vitamin C is possibly that of a nutrient and of a carbon reserve in addition to the part it plays in the phenomena of oxidation-reduction. The last-mentioned rôle represents its real vitamin function.

Von Hausen[7] showed that if the cotyledons of a pea plant were removed after a germination period of five to nine days the plants required ascorbic acid for normal development if nitrogen was present in the form of nitrate. Since no influence of vitamin C was observed when nitrogen was present in the form of $(NH_4)_2SO_4$ it was concluded that ascorbic acid plays a rôle in the reduction of nitrates in plants.

Probably vitamin C functions as a redox system in the plant. In experiments of Virtanen and Saubert-von Hausen[15] with beans it was found that the redox potential in the plant was lowered by vitamin C. It was also proved by Virtanen and Von Hausen[15 16 17] that vitamin C promotes the growth of peas without cotyledons as well as of wheat free from endosperm, in nitrate solution. Between these plants there exists, however, a noteworthy difference. In the pea the influence of vitamin C is strong when the cotyledons have been removed 5 days or still longer after the beginning of germination. But the endosperm must be removed from the wheat germ within a few hours or at least one day after moistening the seed in order to secure a distinct effect from the addition of vitamin C.

On agar media Mitra and Datta[9] found a marked effect of vitamin C on the growth of excised embryos of jute (*Corchorus capsularis*). The effect was found in the presence of both KNO_3 and $(NH_4)_2SO_4$, but was more pronounced on the medium containing KNO_3.

Potatoes are an important source of ascorbic acid in winter time. As winter proceeds the amounts of ascorbic acid in potatoes may decrease substantially. The effects of storage on vitamin C content of potatoes have been investigated *e.g.* by Esselen *et al.*[6], Baker *et al.*[3], Wokes and Nunn[18], Baird and Howatt[2], Barker and Mapson[5]. A general review of literature dealing with vitamin C in the potato was recently published by Allison and Driver[1], who found an increase in ascorbic acid after approximately five months' storage. This phenomenon has not been reported before by other workers for normal storage conditions.

Baird and Howatt[2] found that in New Brunswick values for ascorbic acid were highest in August. They were approximately 150 to 200 mg vitamin C per 100 g of dry weight of potato tissue. In December they decreased to about 50 per cent of the value and by the following May had been reduced to about 25 per cent of the original value. Variation between different varieties reached $\pm$ 25%. No correlation was observed between ascorbic acid content of potatoes and either fertilizer or variety.

According to Hewston *et al.*[8] the ascorbic acid content of raw potatoes varies strongly. The peeled Kathadin variety for example contained ca 36 mg vitamin C per 100 g of dry weight (500 g of fresh weight), or 36 mg in 400 g of cell sap, whereas the ascorbic acid content of the variety Chippewa was 59 mg and of the Green Mountain variety 94 mg per 400 g of cell sap.

METHODS

To investigate the influence of the uptake of vitamin C by the mother tuber under aseptic conditions the same technique was employed as used in earlier work[13 14]. The experiments were made with the early variety Duke of York.

In the experiments with ascorbic acid 25, 50, 100 and 200 mg of vitamin C were added per bottle in 250 ml water. Vitamin C tablets were used. The tablets were placed on watchglasses in a desiccator in the presence of formaldehyde powder. After 48 hours the remainder of the formaldehyde powder was removed from the desiccator and sterile air was sucked through until the tablets were free from formaldehyde. The tablets were added aseptically to sterilized bottles.

The relative amounts of vitamin C in the tubers were indicated by the method of Newton[10]. In this method indicator agar is used containing potassium iodide, soluble starch and glacial acetic acid. Tissue cylinders were removed from potato tubers by means of a cork borer and discs approximately 5 mm in thickness were cut therefrom by means of a razor blade. These were placed immediately on the surface of the indicator agar and the dishes were placed for 24 hours at 5°C in a refrigerator. The diameter of the decolorized zône, formed on the agar medium, is a function of ascorbic acid concentration, if the depth of the agar is constant and the loss of iodine is negligible. It is necessary, therefore, to use dishes with a flat bottom.

EXPERIMENTAL

The influence of ascorbic acid was studied on non-pregerminated

tubers * and on tubers which had been kept for 8 weeks at room temperature and then desprouted. In the experiments with non-pregerminated tubers there were two series with 200 mg ascorbic acid per bottle. The solution of one of these series had been neutralized with NaOH, since it was possible that too high a concentration of ascorbic acid might cause an acid reaction and an eventual harmful effect. The development of the young tubers and the yields of daughter tubers and sprouts of the cultures with non-pregerminated tubers are given in Figure 1.

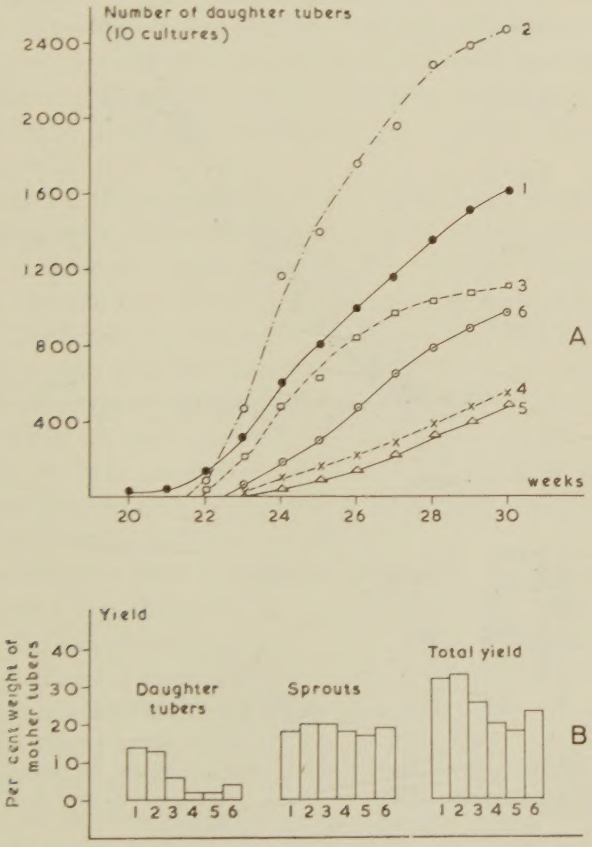


Fig. 1. *1*) Non-pregerminated tubers, culture on water (250 ml); *2*) as *1* + 25 mg vitamin C; *3*) as *1* + 50 mg vitamin C; *4*) as *1* + 100 mg vitamin C; *5*) as *1* + 200 mg vitamin C; *6*) as *1* + 200 mg vitamin C (neutralized).

* For the sake of brevity tubers which had not formed sprouts are called "non-pregerminated tubers", those which had formed sprouts during storage in the dark at room temperature are called "pregerminated tubers".

Although the yield of daughter tubers was slightly reduced and tuber formation was delayed for 10 days by 25 mg vitamin C, the number of daughter tubers was substantially increased as compared with the series without ascorbic acid. But by greater concentrations of vitamin C the yields and numbers of daughter tubers were markedly reduced and tuber formation was delayed for three weeks in presence of 100 and 200 mg of ascorbic acid. The yield of sprouts was

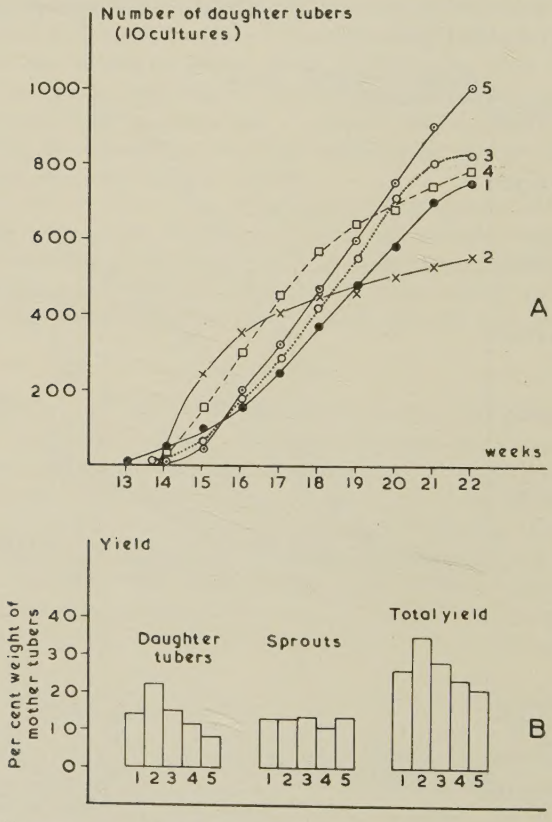


Fig. 2. *1*) Tubers pregerminated for 8 weeks, culture on water (250 ml); *2*) as *1* + 25 mg vitamin; *3*) as *1* + 50 mg vitamin C; *4*) as *1* + 100 mg vitamin C; *5*) as *1* + 200 mg vitamin C.

increased a little by 25 and 50 mg vitamin C and slightly depressed by 200 mg when the solution had not been neutralized. Neutralization of the solution containing 200 mg ascorbic acid (series 6) gave a somewhat greater yield of daughter tubers as compared with the

non-neutralized series 5. In the neutralized series a greater number of daughter tubers developed than in the non-neutralized series.

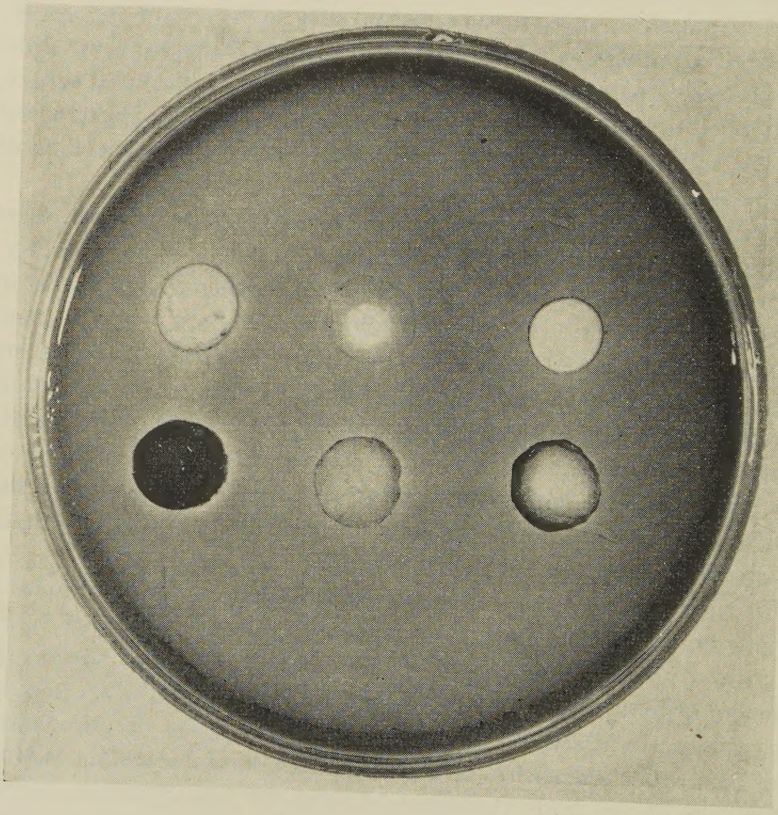
In Figure 2 the results are given for the cultures with pregerminated and desprouted tubers. As compared with the culture without ascorbic acid tuber formation was delayed for some days by vitamin C.

The numbers of daughter tubers were slightly increased by the greater concentrations of vitamin C, most of all by 200 mg. The smallest number developed in presence of 25 mg ascorbic acid, but the yield of daughter tubers was substantially increased in this series. The yield of tubers was decreased by 100 and 200 mg vitamin C, but in a far lesser degree than in the cultures with non-desprouted tubers, probably due to the smaller content of vitamin C of the pregerminated mother tubers. The yields of sprouts were scarcely influenced at all by the ascorbic acid.

At the end of the experiment tissue cylinders of the mother tubers of the series without vitamin C, removed by means of a cork borer, were white coloured, tissue cylinders from mother tubers grown in presence of vitamin C were yellow coloured, the more yellow as more vitamin C had been present in the solutions. The flesh of the tubers from the series with 200 mg vitamin C proved to be as yellow coloured as the tissue of tubers kept at 2°C. At the end of the experiment it could be shown, moreover, by means of the method of N e w t o n [10] that small discs cut from corresponding parts in the mother tubers contained increasing amounts of vitamin C the more vitamin had been present in the solution.

By the same method it could be shown that non-pregerminated tubers grown for 12 weeks on glucose solutions contained more vitamin C than those grown in pure water. Figure 3 shows the reaction from discs taken from the central part of (1) a tuber kept in the refrigerator at 2°C, (2) a tuber placed for 12 weeks on pure water and (3) a tuber placed for 12 weeks on 3% glucose solution. Before taking the photograph, the discs of the upper row were removed to show the decolorized zônes formed in the agar. As can be seen, the discs of the potato kept at 2°C (at the left) and of the potato placed on the glucose solution (at the right) contained more ascorbic acid than the disc of the potato placed on pure water (in the middle). The discs of the lower row were not removed. The disc on the left from the tuber kept at 2°C contained much starch which was dark coloured

owing to the potassium iodide present in the agar. Starch was present in a small amount in the disc on the right, from the tuber placed on glucose, and absent in the middle disc from the tuber placed on water. Probably there is a connection between the loss of vitamin C and the conversion of starch into sugars in the same way as there is a close correlation between ascorbic acid and sucrose changes in different varieties of potatoes as found by Barker[4], who on the basis of this correlation postulated a close relationship between ascorbic acid and respiration.



1 2 3

Fig. 3. Explanation in text.

The greater amount of vitamin C in tubers grown on glucose solution as compared with those grown on water may be caused by the retarded growth and by a diminished conversion of starch into

sugars. Another (less probable) possibility is that the glucose in the sterile solutions has stimulated the formation of ascorbic acid. In this connection the work of Ray [11] may be recalled, who found that in sterile media addition of 5% glucose, 5% fructose, 5% galactose, or 5% mannose resulted in the formation of ascorbic acid in excised embryos of peas. In these cases the hexoses acted as a precursor of ascorbic acid. Especially the addition of mannose resulted in an abundant formation of vitamin C.

SUMMARY

In experiments with tubers of the variety Duke of York, which had not formed sprouts during storage, the number of daughter tubers was increased in cultures containing small amounts of vitamin C. The yields and numbers of daughter tubers were markedly reduced by greater concentrations of vitamin C and tuber formation was delayed.

In cultures with desprouted tubers the formation of daughter tubers was also delayed by vitamin C, but in a far lesser degree. In these cultures the numbers of young tubers were slightly increased by the greater concentrations of vitamin C, most of all in presence of 200 mg. The yield of tubers was substantially increased in presence of 25 mg ascorbic acid.

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INTRODUCTION

Generally leaves and seeds are rich in vitamin B_1. In contrast with the embryo of higher plants, which is auxoheterotrophic during only a part of its development, the root is always more or less auxoheterotrophic, and must therefore depend on the leaf, which is able to synthesize all the necessary growth factors, including vitamin B_1.

From the studies of Bonner and Devirian[5] and Bonner[6] with roots growing in pure culture, it can be concluded that some vitamin synthesis by roots is possible but it never is significant. Different kinds of roots vary in their ability to synthesize vitamins.

A marked effect of vitamin B_1 on root development was shown by Kögl and Haagen Smit[7] with embryo cultures of *Pisum*. The importance of vitamin B_1 for young roots in root cultures has been shown by several investigators (Robbins and Bartley[10], White[23], Bonner and Addicott[3]). Roots of tomato do not require the whole molecule of thiamin, but only the thiazole fraction in contrast to roots of peas which require both fractions of thiamin: thiazole and pyrimidine. The action of vitamin B_1 has been studied by Addicott[1] in regard to its effect upon the cytology of the root of *Pisum*. Vitamin B_1 stimulates primarily the meristematic activity of roots but does not affect cell elongation. Its action is associated with that of auxin. In pea cultures, supplied with various growth substances Von Hausen[11] found that vitamin B_1, biotin and the vitamin B complex had a slightly stimulative effect on the growth when the cotyledons had been previously removed. The influence of vitamin C, however was far superior. Recently Schopfer[16] has given a critical review of the literature dealing with the rôle of vitamin B_1 in lower and higher plants.

Meiklejohn[9] made a study of the vitamin B_1 content of potatoes, using the mould *Phycomyces blakesleanus* as a test organism. The use of this

mould for estimating vitamin B_1 was first proposed by Schopfer [13 14] and further worked out by Schopfer and Jung [15]. The method employed by J. Meiklejohn was an adaptation, for use with plant material, of the method for blood estimation worked out by A. P. Meiklejohn [8] and Sinclair [21 22]. She found that during the growing season the vitamin B_1 content of tubers increases continuously up to the time of harvest. Mature tubers of the variety Majestic had a vitamin B_1 content of about 140 μg per 100 g fresh material. In winter the vitamin apparently remains in the tuber till sprouting begins, when it moves out of the tuber into the sprouts. As a consequence the vitamin B_1 content of stored tubers falls during the winter and spring when white sprouts are formed during storage. A much higher content of vitamin B_1 was found in sprouts than in the middle part of the tuber.

METHODS

To investigate the influence of the uptake of vitamin B_1 by the mother tuber under aseptic conditions, the same technique was employed as described in earlier work [18]. A solution containing vitamin B_1 was allowed to be absorbed by the mother tuber by means of a lamp-wick which hung in the culture solution and which had been inserted with the other end into the mother tuber.

The vitamin B_1 content of tubers, sprouts and solutions was determined according to the method of Meiklejohn [9]. The growth of *Phycomyces blakesleanus* was compared in cultures containing potato tissue, or sap of potatoes, or culture solution, with a dose-response curve obtained from a series of control cultures containing known amounts of the vitamin.

The experiments were carried out with the early variety Duke of York.

EXPERIMENTAL

At the start of the experiment with the first lot of potatoes (1 October 1954)the vitamin B_1 content was estimated in a number of potatoes. Cores were taken from the heel end and the rose end separately for determinations. It was found that the heel end contained 95 μg vitamin B_1 per 100 g fresh material and the rose end 84 μg per 100 g (mean value of 20 determinations).

The tubers of the first lot had not yet formed sprouts and were placed on bottles containing 150 ml solution. Before sterilization for 1 hour at 120°C the bottles contained 0, 50, 200 and 400 μg vitamin B_1. After sterilization only values were found of 0, 19, 55 and 75 μg per bottle. Thus a large percentage of the vitamin had been destroyed by sterilization. For that reason greater amounts of vitamin B_1 were used for the second lot of tubers.

This lot was kept first for a long period (from 1 October '54 to 4

February '55) in the dark at room temperature. After desprouting the whole lot had lost 33.7 per cent of its weight during this period. At this time values were found of 98 μg vitamin B_1 for the heel end and 99 μg for the rose end per 100 g of fresh material. Taking into account the loss of material during germination (most of it as water) these values correspond with 65.0 and 65.6 μg per 100 g of original tissue. The sprouts had a much higher content of vitamin B_1 than the tubers, giving a mean value of 172 μg per 100 g fresh material (10 determinations).

The desprouted tubers were placed on bottles with 150 ml solution. Before sterilization four series of fifteen bottles were provided with 250, 500, 1000 and 2000 μg vitamin B_1. After sterilization for 1 hour at 120°C the bottles contained 49, 180, 262 and 1155 μg vitamin B_1 (mean values of 3 bottles of each series).

The growth response of the first lot of potatoes (here called "non-pregerminated tubers") on the increasing amounts of vitamin B_1 is seen in Figure 1. The number of young tubers was increased temporarily in presence of 19 μg of vitamin B_1, but at the end of the experiment the number was practically the same as in the control series. The weight of daughter tubers, however, was markedly increased. In presence of 55 μg vitamin B_1 there was a definite increase in the number of daughter tubers. There was a somewhat smaller increase in weight of daughter tubers as compared with the smaller addition of vitamin B_1. The highest weight of sprouts and young tubers, however, was found in presence of 55 μg vitamin B_1. The formation of young tubers was delayed for some weeks in presence of 75 μg vitamin $B_1$and the number of daughter tubers was only increased in the last period of growth. The weight of daughter tubers was further decreased as compared with the series supplied with 55 μg vitamin B_1. The weight of sprouts was not increased by the addition of vitamin B_1.

The effect of the increasing amounts of vitamin B_1 on the growth response of the potatoes which had been kept for a long period in the dark and which had been desprouted before placing them upon the bottles (here called "pregerminated tubers") is given in Figure 2.

The number of daughter tubers was increased in presence of 49 and 180 μg vitamin B_1, especially notable in the latter case. The number of daughter tubers, however, was markedly reduced in presence of 262 and 1155 μg vitamin B_1. The weight of young tubers

was increased by all additions of vitamin B_1, but mostly in presence of 49 μg vitamin B_1, decreasing gradually in presence of the larger amounts. As in the former experiment the weight of the sprouts was not increased by the additions of this vitamin. The greatest weight

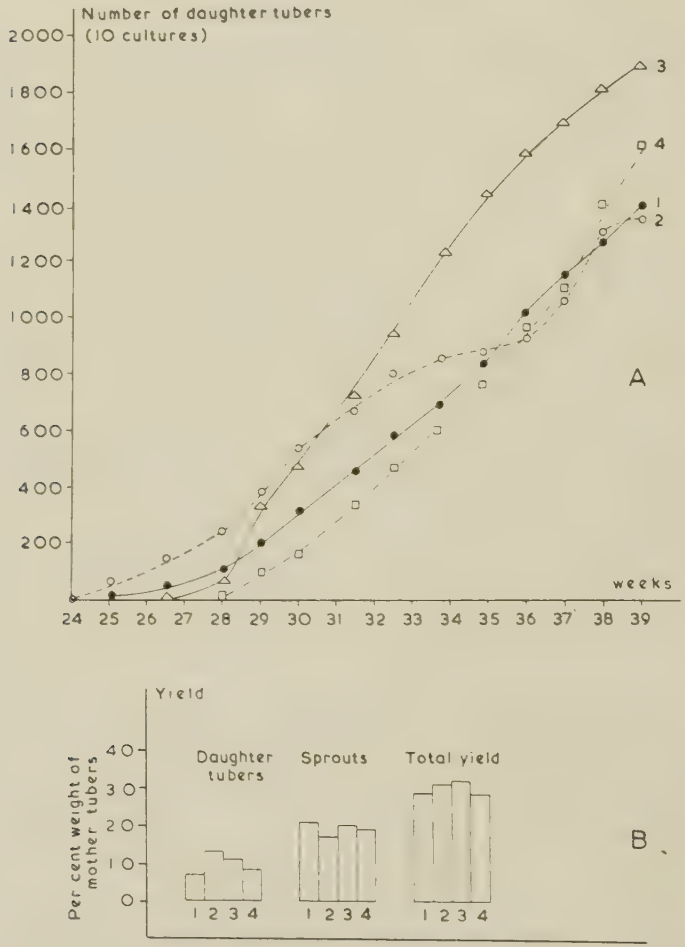

Fig. 1. *1*) Non-pregerminated tubers grown on water (150 ml); *2*) as *1* + 19 mg vitamin B_1; *3*) as *1* + 55 mg vitamin B_1; *4*) as *1* + 75 mg vitamin B_1.

of sprouts and tubers was found in presence of 49 and 180 μg vitamin B_1.

At the end of the experiment borings of the tubers were made at

the heel and the rose end for determinations of vitamin B_1. As in the former determinations samples of 0.1 and 0.15 g were used per flask. The vitamin B_1 content of these samples, however, was too low for a good comparison with the standard series, so that it was impossible

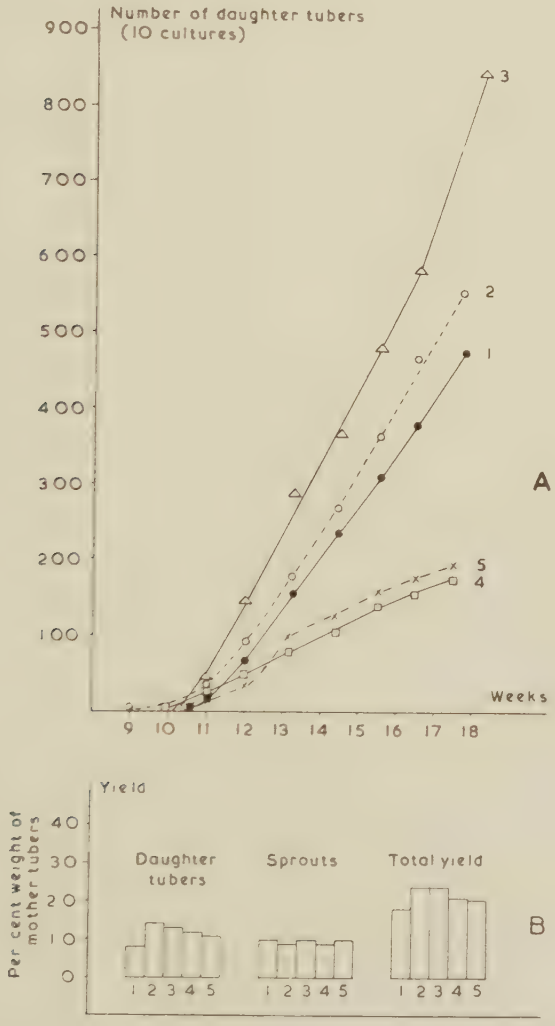

Fig. 2. *1*) Tubers kept for 4 months in the dark at room temperature, desprouted, grown on water (150 ml); *2*) as *1* + 49 mg vitamin B_1; *3*) as *1* + 180 mg vitamin B_1; *4*) as *1* + 262 mg vitamin B_1; *5*) as *1* + 1155 mg vitamin B_1.

to calculate the absolute contents of the vitamin B_1. For that reason only the dry weights of the mycelium are given in Table I.

TABLE I

Mean weights of dry mycelium in mg per sample taken from the rose and heel end of the tubers at the end of the experiment

Vit. B_1 μg per bottle	Weight of sample g	Rose end		Heel end	
		Mean weight of dry mycelium (mg)	Sum	Mean weight of dry mycelium (mg)	Sum
0	0.1	6.0		4.6	
0	0.15	6.6	12.6	5.7	10.3
49	0.1	6.1		5.0	
49	0.15	6.8	12.9	5.8	10.8
180	0.1	11.4		3.5	
180	0.15	8.7	20.1	8.8	12.3
262	0.1	8.5		5.8	
262	0.15	10.5	19.4	8.5	14.3
1155	0.1	20.0		12.5	
1555	0.15	27.5	47.5	12.2	24.7

On the whole a greater weight of the mycelium was found as more thiamin was present in the bottles and greater values were found in the rose end than in the heel end. The mycelium weight found for tubers supplied with 1155 μg vitamin B_1 corresponded with a vitamin B_1 content of < 35 μg per 100 g tissue. A greater value was found when the rest of the tubers was minced and boiled in water for ca. 30 minutes, pulverised in a mortar, water added up to 500 ml, filtered and 0.9 ml of the solution used for the determination of vitamin B_1. Using this method a vitamin B_1 content of 52 μg per 100 g tissue was found.

It is remarkable that during the sterilization of the bottles an important part of the vitamin B_1 was destroyed. Schopfer [12] found that this vitamin is very resistant to prolonged heating at 120–130°C, but in an alkaline medium an important part of the vitamin B_1 was destroyed by sterilization for 30 minutes at 120°C.

As it was possible that the reaction of the solution in the bottles would turn alkaline during the sterilization from contact with the glass of the bottles, a number of solutions was made in the bottles by adding 0, 250, 500, 1000 and 2000 μg vitamin B_1 to 150 ml water and pH determinations by means of the glasselectrode were carried out after sterilization. The pH values were: 7.90, 7.14. 6.54, 6.39 and

6.24, respectively. The reaction, therefore, was indeed alkaline in the absence of vitamin B_1, but turned more acid as more vitamin B_1 was added, possibly owing to the presence of a carrier. The partial destruction of the vitamin B_1 during sterilization cannot therefore be caused by an alkaline reaction of the solution.

Influence of vitamin B_1 and vitamin C in combination with a growth substance

Data on the effect of growth substances and vitamin C on tuber and sprout formation of potatoes aseptically grown in the dark have been published in previous papers [19] [20]. The combined effect of vitamins and a growth substance was investigated in the following experiment. Tubers which had been kept in the dark for two months and which had been desprouted were placed on solutions of the following composition:

1. Water (250 ml)
2. As 1 + 50 μg vitamin B_1 + 25 mg vitamin C
3. As 2 + 0.5 mg IAA *
4. As 1 + 50 mg vitamin C + 0.5 mg IAA
5. As 1 + 0.5 mg IAA.

As is shown in Figure 3, the number of young tubers increased in the course of the experiment when vitamin B_1 and vitamin C or vitamin C alone had been supplied in addition to 0.5 mg IAA, but at the end of the experiment the numbers were not higher than in the control series. The weight of the young tubers was increased by vitamin C plus vitamin B_1. In presence of 0.5 mg IAA the weight of the daughter tubers was not further increased by vitamin C or by vitamin B_1 plus vitamin C.

The yield of sprouts was neither increased by a combined addition of vitamin B_1 and vitamin C, nor by these vitamins in combination with IAA. A slight increase in the yield of sprouts was obtained by 0.5 mg IAA without vitamins.

The increase in total weight of the sprouts and tubers caused by the vitamins or IAA, or by the combination of vitamins and IAA, is chiefly the result of an increased weight of the daughter tubers. The greatest increase was obtained by 0.5 mg IAA without vitamins.

* The sodium salt of β-indolylacetic acid.

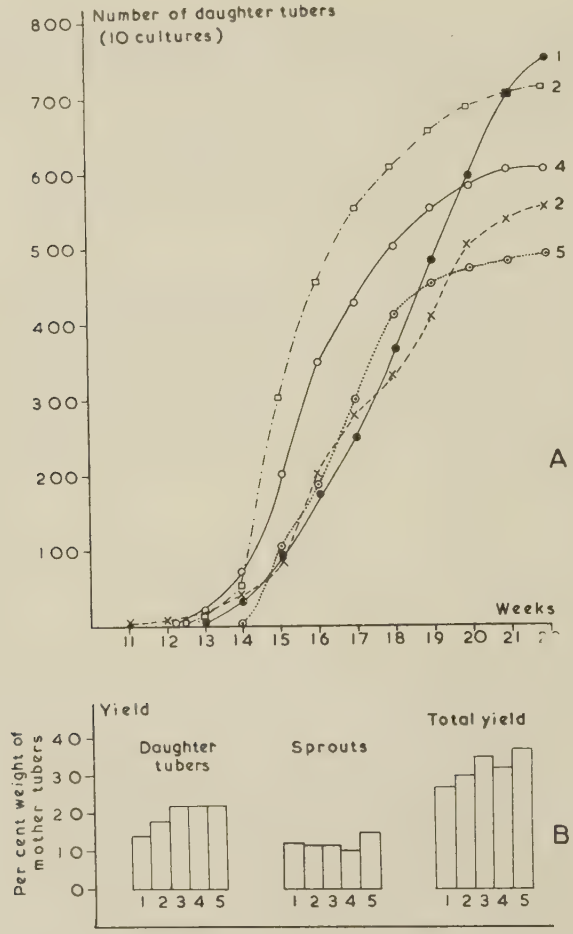

Fig. 3. *1*) Tubers kept for 8 weeks at room temperature in the dark, desprouted, grown on water (250 ml); *2*) as *1* + 0.25 mg Vitamin B_1 + 25 mg vitamin C; *3*) as *2* + 0.5 mg IAA; *4*) as *1* + 50 mg vitamin C + 0.5 mg IAA; *5*) as *1* + 0.5 mg IAA.

DISCUSSION

In the experiments with vitamin C [20] it was found that additions of this vitamin in small concentrations delayed the formation of of young tubers for one week in the cultures with pregerminated tubers. In presence of small amounts of vitamin B_1 and IAA, however, this "delaying effect" of vitamin C is compensated more

or less. In the cultures with pregerminated tubers a "delaying effect" of vitamin B_1 was not found with the concentrations used in the experiment (Figure 2). As vitamin B_1 is of great importance for the development of the roots [1 2 3 4 5 6 7 10 23], it is possible, however, that a shortage of vitamin B_1 in desprouted mother tubers may be responsible for a weaker development of roots and indirectly for a weaker development of sprouts, a condition which is correlated with premature tuber formation [17].

On the whole, tuber formation in the cultures with desprouted tubers was only slightly, or not delayed by loss of vitamins and growth substances in the period of pregermination.

The most important factor for premature tuber formation, therefore, seems to be the fact that the highest proportion of soluble carbohydrates to soluble organic nitrogen in the tubers and in the sprouts, which is correlated with the development of young tubers, is reached at an earlier moment when pregerminated tubers are used, as compared with non-pregerminated tubers [18]. Since tubers produce more sugars at a low temperature, it is most probable that a high ratio of soluble carbohydrates to soluble nitrogen in the tubers and sprouts is reached earlier when the temperature is kept low during the first development of the plant.

SUMMARY

In cultures with tubers which had not formed sprouts during storage the number of daughter tubers was increased in presence of 55 μg vitamin B_1 and the weight of daughter tubers was increased in presence of 19 μg vitamin B_1. The formation of daughter tubers was delayed for some weeks in presence of 75 μg vitamin B_1. In cultures with tubers which had been kept for 2 months in the dark at room temperature and which were desprouted, the number of daughter tubers was substantially increased in presence of 49 and of 180 μg vitamin B_1. The number of tubers was greatly reduced by large amounts of vitamin B_1.

The results are discussed in connection with the problem of premature tuber formation. It is evident that in the normal growing plant vitamins play an important rôle on the development of the daughter tubers.

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CALIBRATION OF SOIL TEST METHODS FOR THE DETERMINATION OF PHOSPHATE AND POTASH STATUS

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INTRODUCTION

Intensive agriculture requires accurate advice on fertilizer requirements. If the advice is based on soil analyses much attention has to be paid to the agricultural "calibration" of these analyses.

The correlations between the analyses and the behaviour of the crops have to be established under normal farming conditions. Inevitably these conditions vary and calibration must therefore be performed on various soils and under various soil conditions. The following is an account of such calibration work carried out in the Netherlands.

METHODS

The general procedure in an investigation of this kind consists of the laying out of a series of field trials, on one soil group, all carrying the same crop. Such experiments are of one year's duration and are completed by further experiments which are continued for several years. The short-term experiments give the most direct information on the value of soil analyses. The long-term experiments make possible a comparison between the different crops of one rotation, and also the determination of changes in soil status under continued yearly fertilizer dressings of different magnitude.

Frequently the lay-out of the experiment is influenced by previous information. For example it is known that the availability of potash to the crop depends both on the potash status and on the lime status

of the soil. Care should therefore be taken that these factors are correctly related in the investigation.

The trial fields must be selected in such a way that there is considerable variation in the potash values as well as in the other factors mentioned. The selection of the fields therefore requires preliminary soil analyses. If extensive soil analyses, in relation to agricultural practice, have previously been carried out on soils in the region where it is intended to establish the experiments, then data from such analyses may be a valuable help in selecting the trial fields.

The number of trial fields required for an adequate experiment depends on the number of factors which are expected to be operative, the extent of the correlations between these factors and the extent of their influence and interactions. Experience has taught us that at least ab. 30 trial fields are necessary for a limited area.

Care should be taken that the proportion of the various categories of trial fields is relatively equal, *i.e.* as far as possible equal numbers of fields which are poor, moderate, or rich in potash (and also poor or rich in lime, clay, or humus), should be selected even if some categories are rare in the particular region being investigated. An effort is also made to avoid correlations between the principal factors to be studied. If, for example, there is an evident correlation between potash and clay content, then an attempt is made to select the trial fields in such a way that both at high and low potash content there is ample variation in the other factor and vice versa. Another aim is to acquire an even distribution from a geographical point of view.

Fig. 1 shows an example in which the fields intended for experimentation have been selected from the results of previous soil analyses.The choice was made from the graphs obtained by plotting the potash value against both the lime content and clay content (particles less than 16 μ) and also the two latter against each other. If 3 factors are involved, the combinations selected to be included in an experiment will, therefore, have to be evenly distributed over a cube.

The lay-out of the trial fields can be simple. In the above-mentioned experiments concerning potash, each field contained 10 plots, each of 25 square metres. Four plots received no dresssing of potash; of the remainder, duplicate plots received 60, 150, 300 kg K_2O per

to as sufficient of potash. It is usual to express the yield obtained in the absence of added fertilizer as a percentage of the maximum yield which can be obtained on the same field under optimum conditions with regard to the factor examined. Experience has shown that percentage differences are more suitable than absolute ones. This relative yield is a measure of requirements for the nutrient in ques-

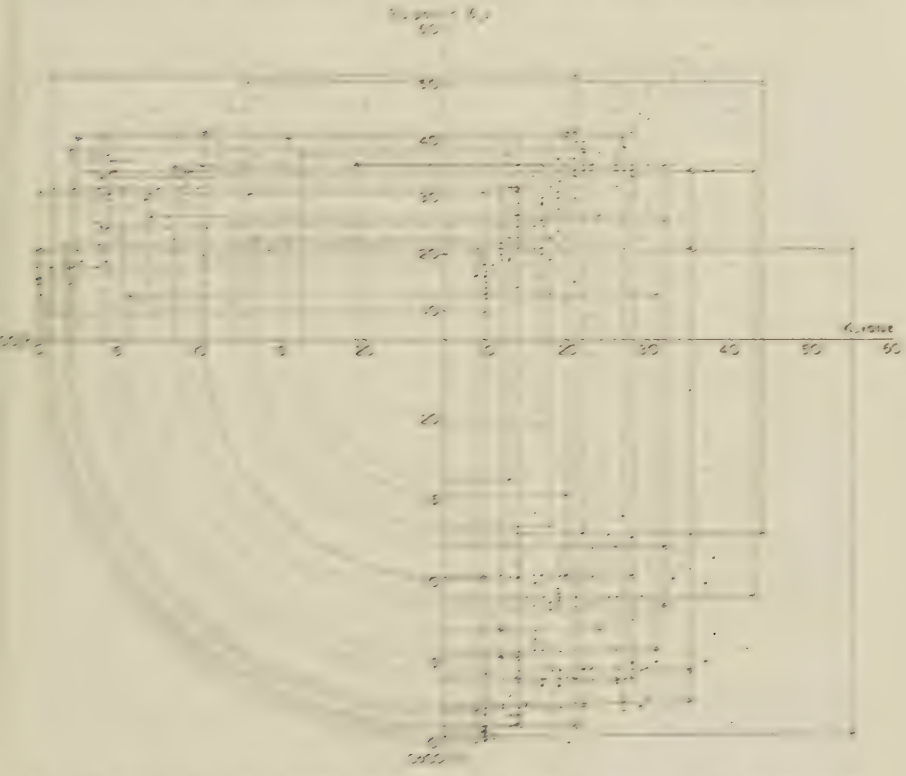


Fig. 1. Relation of local fields from regional soil analyses after allowing for correlations between various factors and among an even distribution. Dots represent the results of soil analyses. Circles represent selected trial fields.

tion. A disadvantage of this method is, however, that the highest yield obtained does not always agree with the theoretical maximum capacity of the soil. The effect of even large additions of the fertilizer often remains below this level. This discrepancy is especially prevalent on soils having high nutrient-immobilizing power and under low rainfall conditions, and also on grassland where the highest

possible productivity of the sward is not reached by a single fertilizer dressing. In addition, the determination of relatively small differences in yields demands a high level of accuracy in the experiments.

It is also very important that other properties of the crop which may be indicative of the availability of soil nutrients should be used for this purpose. The assessment of the content of a particular nutrient in plants, especially in the case of a rather young crop, from visible deficiency symptoms which can be expressed on an arbitrary scale 1–10 (see Ref. [2 9]), or from qualitative properties, such as starch content of potatoes or grain weight of cereals *etc.*, is often more accurate than the determination of differences of yields. Experience has shown that they are generally sensitive indicators of nutrient deficiency. A great advantage of the use of these indications is that they often already demonstrate deficiency symptoms even when the nutrient concerned still is available in a concentration sufficient for optimum yields. This results in many more data being available for statistical analysis. A disadvantage of the use of these properties of the crop is of course that before there can be application of the results verification with the yields is necessary.

The results of the field trials as measured by yields, chemical composition *etc.* are related to the nutrient status of the soils. Each field trial then gives one result in the experiment. The effect of some factors might be established by the aid of a linear regression analysis.

Experience shows, however, that the relations are mostly non-linear. For this reason mathematical calculation is generally abandoned and a graphical treatment is adopted. It is possible to test statistically the reliability of the results obtained in this way (Ferrari [1], Ferrari and Sluijsmans [2]).

The fact that various properties of the crops can be used for calibration of the soil tests implies that much more simple experimental procedures may be followed in special cases. The possibility of field experimentation was limited to a great extent during World War II. Nevertheless an extensive investigation was conducted throughout the country by taking numerous crop and soil samples simultaneously from small plots of $^1/_4$ square metre each.

Other investigations which have contributed to the calibration of methods of soil analyses are the so-called regional soil fertility analyses. In this case experimental fields consisting of a number

of plots are not laid out. Only single plots are used to which varied treatments are not applied, but of which the crop yield is determined and soil samples are taken. A statistical analysis of all the factors affecting the yield is then carried out; this is named *polyfactor analysis*. The relation between soil factors and yield and the interrelations of the former can be established (Ferrari [1]).

Evaluation of soil analyses by means of pot experiments may be useful in special cases. Experimental series performed in several clay soil regions in some years did not yield completely equivalent results. In an extensive pot experiment, soils from different regions have been studied simultaneously and under the same climatological and experimental conditions. Information as to whether differences in response to potash are due to differences between the soils has been obtained. In this way valuable information on the utility of soil analyses can be obtained. The calibration of analyses must, however, be restricted to the results from the field trials.

CORRELATION BETWEEN SOIL ANALYSIS AND CROP REACTION

A. *Short-term experiments*

1. Determination of the phosphate status of permanent grassland by soil analysis

The method in use in the Netherlands is to extract the soil sample (on grassland the 0–5 cm layer) with a 1% solution of citric acid. The *P-citr value* obtained from this treatment indicates the amount of P_2O_5 as mg per 100 gram of air-dry soil.

Series of experiments (20–22 experiments per series) were laid down in the years 1939–1941 on three soils in the neighbourhood of the town of Groningen. This investigation was repeated after the war on a much larger scale throughout the country. During 1947 and 1948, 330 field trials were laid down with the aid of the Agricultural Advisory Service. These experiments were divided into series, according to soil group and geographical situation. The main groups of soils investigated were peat, marine clay, river clay, loess and sand soils respectively.

The field trials consisted of 12 plots, each 5×5 metres. Four of these plots did not receive any dressing with phosphate, the others were dressed in duplicate with 20, 50, 90 and 140 kg P_2O_5 per ha. (in the form of double superphosphate). Nitrogen was added as

ammonium nitrate limestone (50 kg N per ha) and potash in different amounts according to the need of the fields. Only one mowing was harvested.

The average yields of the plots without phosphate dressing as a percentage of the maximum yield obtained from plots with adequate fertilizer, on the same field, have been used as a measure of the need for application of phosphate.

It is assumed here that the yield of a field with a low phosphate status will, when adequately dressed with phosphate, be of the same order as that of fields having a much higher phosphate level, so that the yields in both cases can then be put at 100. This is certainly not true if fields highly deficient in phosphate are included in the investigation. The present investigation has, however, been restricted to fields in normal condition. It could be proved that, under such conditions the assumption was justified.

The P_2O_5 content of the dry matter of grass grown on plots receiving no phosphate dressing has been used for the same purpose. However, a correction had to be made for the varying N-contents of the grass of the different field-trials. By means of the definite ratio observed between the N and the P_2O_5 contents of the grass, it was possible to adjust the P_2O_5 contents to those for grass having an equal N-content. In consequence, variations arising from differing stages of physiological development and different species composition are largely eliminated. These adjusted values for the P_2O_5 content of the grass proved to be a sensitive measure of the availability of soil phosphorus.

Some examples may give a better picture of the results obtained.

A series of 33 experiments was performed in 1947 on a clay soil in the northern and north-western part of the country and another series of 29 field-trials in 1948 in the western and south-western part. Relative yields in both series have been plotted against P-citr values in Fig. 2. The results are very satisfactory and indicate that in both years the yield progressively decreased where the P-citr value fell below 40. With P-citr values higher than 40, only small increases were observed when additional phosphate was added.

In another case concerning a series of 40 field trials made in 1947 on peaty grassland the result was less satisfactory (Fig. 3). The P_2O_5 contents of the dry matter of the grass were, however, much better

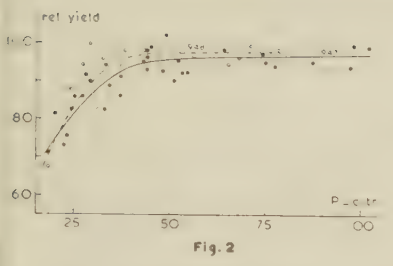


Fig. 2

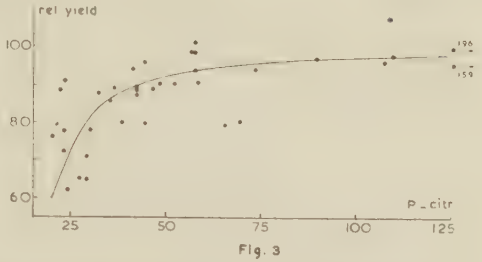


Fig. 3

Fig. 2. Relation between P-citr value and relative yields for marine clay soils for 1947 (dots) and 1948 (circles).

Fig. 3. Relation between P-citr value and relative yields on peaty soil (1947).

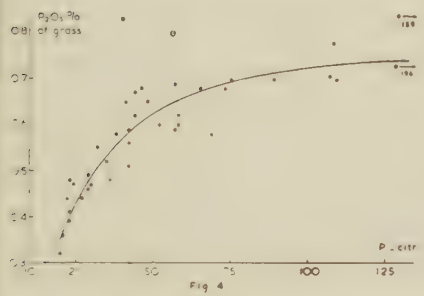


Fig. 4

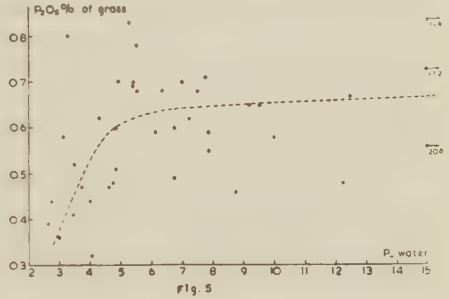


Fig. 5

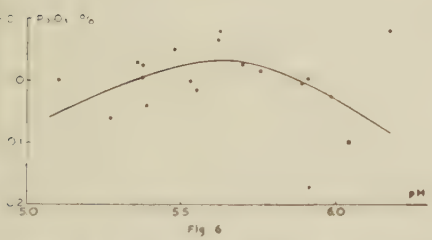


Fig. 6

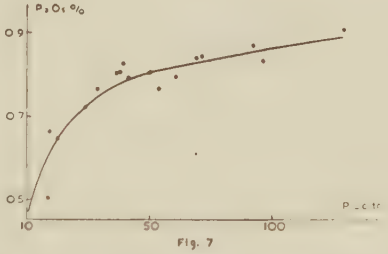


Fig. 7

Fig. 4. Relation between P-citr value and P_2O_5 content of the dry matter of grass (corrected to N = 1.8%) grown on peaty soil not dressed with phosphate (1947).

Fig. 5. Values for the P_2O_5 contents of the dry matter of grass as used in Figure 4 plotted against *P-water* (soil sample extracted with warm water, 50 °C).

Fig. 6. Relation between pH of the soil and P_2O_5 content of grass (positive or negative differences from the average content for sandy soil, after elimination of the influence of variations in P-citr value and differing N-contents of the grass (1941).

Fig. 7. Relation between P-citr value and P_2O_5 content of grass grown on sandy soil, after elimination of the influence of differing soil pH and variation in the N-content of the grass (1941).

correlated with the P-citr values (after elimination of the variations due to different N-contents) thereby indicating that the less pronounced correlation of relative yields was not due to a defect in the method of soil analysis (Fig. 4).

In some cases the correlation could be improved by taking other factors into account. The scatter of the points observed in some graphs proved to be partly due to other factors such as pH of the soil and humus content. An example of the first is given by an experimental series for 1941 on sandy soil. In this case the positive or negative difference between the actual values for P_2O_5 content of the grass after elimination of the variations due to difference in N-content and the values appropriate to the corresponding P-citr values according to the smooth curve through all the points have been plotted against pH (Fig. 6). For this procedure to be valid it is necessary that there should be no correlation between P-citr and pH. The positive deviations of P_2O_5 contents occurred at moderate pH values and negative deviations at relatively low and high pH. A similar relationship has been noted repeatedly for different kinds of soil. The correlation of P_2O_5 content of grass with P-citr value in this series is shown in Fig. 7.

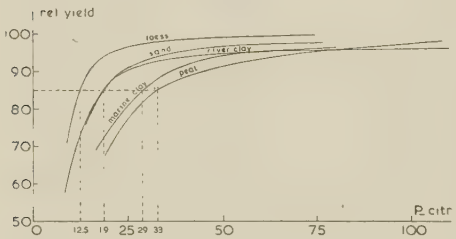


Fig. 8

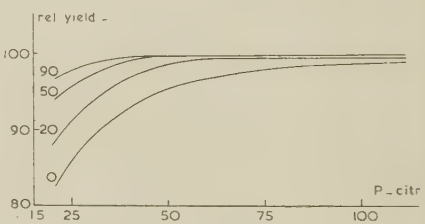


Fig. 9

Fig. 8. Relations between the averaged values for varying numbers of years, for P-citr value and relative yields as determined for marine (3 years) and river (1 year) clay soils, loess soil (2 years), peaty soil (2 years) and sandy soil (3 years).

Fig. 9. Relation between P-citr value and relative yields at different levels of phosphate application (kg P_2O_5 per ha) for sandy soil (1941).

These and similar results obtained for other series in different years have been averaged (Fig. 8). It is clear from this that the agronomical value of P-citr values differs from soil to soil. A value of 33 on peaty soil proves to be equivalent to 19 on river clay and sandy soil, to 29 on marine clay and to 12.5 on loess.

In addition to such considerations fertilizer advice is based on results derived from the effects obtained with different amounts of P-dressing. The correlation between P-citr and relative yields for different P-dressings, obtained in the above mentioned series of field trials on sandy soil in 1941, is shown in Fig. 9.

As the effect of small additions of phosphate is relatively large by comparison with the effect of differences in P-citr value, it is concluded that the effect of freshly added fertilizer phosphorus is much greater than the effect of an equal amount of soil phosphorus.

As a result, the following advisory scheme (Table I) has been derived from the experimental results. It has been put forward in consultation with grassland specialists. In some ways the work is incomplete as it only relates to results obtained for the first mowing of grasslands. The reaction of grassland to phosphorus manuring at mid-summer is certainly less than that in spring.

TABLE I

Advice regarding phosphate manuring of grassland based on the P-citr values obtained for grassland mown once and used for grazing afterwards

P-citr value					P_2O_5 dressing
Peaty soil	Marine clay	River clay	Sandy soil	Loess	in kg/ha
<36	<31	<21	<21	<16	150—100
36—55	31—45	21—35	21—35	16—25	80—60
56—80	46—65	36—60	36—60	26—45	50—40
81—120	66—100	61—90	61—90	46—65	30—20
>120	>100	>90	>90	>65	0

With other cropping treatments the following deviations from this scheme are recommended:

1) When continually used for grazing 20 kg P_2O_5 per ha less dressing each year and with very intensive use only 10 kg per ha less each year.

2) When mown twice and used for grazing afterwards an additional supply of 30 kg P_2O_5 per ha and with very intensive use an additional supply of 40 kg per ha.

3) When mown exclusively (*e.g.* for grass drying) an additional supply of 110 kg P_2O_5 per ha yearly.

It is interesting to note that another method, in which the content of P_2O_5 soluble in water at 50°C is determined, adopted in the Netherlands, proved to be impracticable in some cases. Fig. 5 shows the same P_2O_5 contents of the dry matter of grass as presented in Fig. 4 plotted against this value. Nevertheless this method was useful in other cases. A special disadvantage of this method is the large variability over a period of time.

It has been mentioned that simplified experiments were performed during the war. Samples of soils and plants taken simultaneously from the same plot yielded valuable results. Fig. 10 shows the relation between the P-citr value and the P_2O_5 content of grass from grassland on river clay soils in the central region of the country. Fig. 11 similarly shows the relations between the P-citr value and the P_2O_5 content of young rye grown on river clay soil.

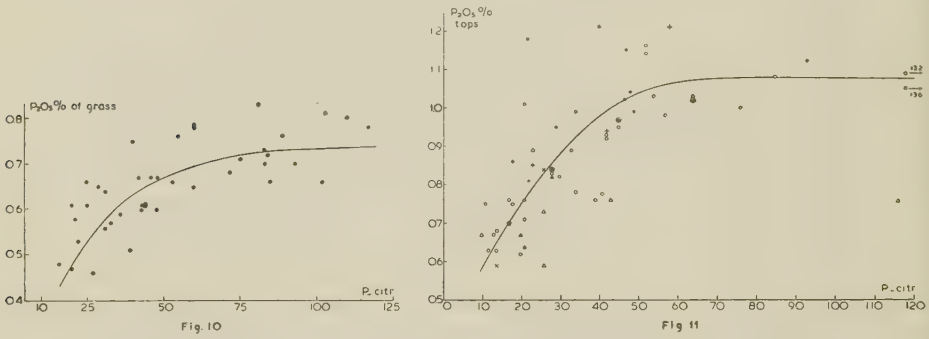


Fig. 10. Relation between P-citr value and P_2O_5 content of grass grown on marine clay soils (1943) using the sampling method, without corrections for interactions of other factors.

Fig. 11. Relation between P-citr value and P_2O_5 content of young rye grown on river clay soil (1943) using the sampling method, without corrections for interactions with other factors.

2. Determination of the potash status of arable land from soil analyses

The discussion of the problem is restricted to investigations carried out on clay soils. It will be shown that the problem is rather complicated but that, nevertheless, useful results have been obtained.

Experiments on four series of experimental fields carrying potatoes were performed in different years and in different parts of the marine clay region. The setting up of such an investigation has been discussed before (Fig. 1). The results from such a series of experiments will be discussed below.

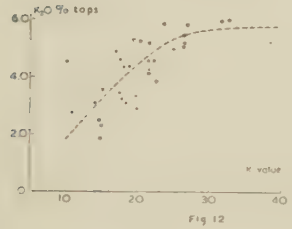
The relation was studied between the *K-value* * and the increases in yields of tubers resulting from potash dressings the K_2O-contents

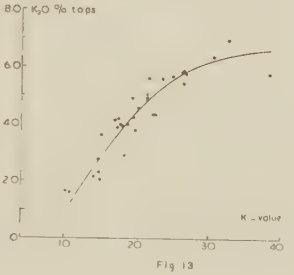
* This *K-value* indicates the amount of exchangeable K_2O extracted with 0.1 *N* HCL as mg per 100 g of air-dry soil.

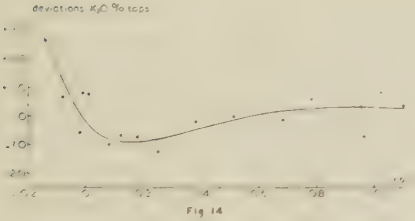
of tops of fairly young plants and of tubers under-water-weights of tubers, actual yields and in some cases by vigour scoring in addition.

The relation between the K-value and the K_2O-contents of the tops of the potato plants generally gave the most obvious indications. The results obtained have been checked, however, by the other observations and have been related to them.

In agreement with the results of former investigations the availability of potash proved to be dependent on three soil factors, namely the K-value, the lime status, and the clay content (particles $< 16\ \mu$). The effect of each of these factors has been determined in turn, those factors having the largest effects being analysed first. According







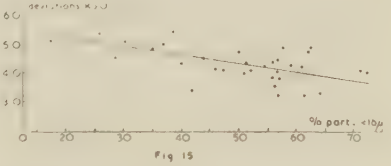


Fig. 12. Relation between K-value of the soil and the K_2O content of potato tops for marine clay soil (1950).

Fig. 13. Relation between the K-value of the soil and the K_2O content of potato tops after elimination of the influence of variations in lime status and content of particles $< 16\ \mu$ for marine clay soil (1950).

Fig. 14. Relation, for a low K-value < 20, between the lime status (lrb) and K_2O content of potato tops (grown on soil not dressed with potash) measured as deviations of the values from the average curve after elimination of the influence of variation in the content of particles $< 16\ \mu$ (average content = 50%).

Fig. 15. Relation between content of particles $< 16\ \mu$ and K_2O content of potato tops, as deviations from the mean, after the influence of variation in K-value and lime status have been eliminated.

to this method, called polyfactor analysis, the influence of the K-value (being the most important factor according to preliminary determinations of correlations) has been provisionally determined by a free-hand curve (Fig. 12).

This having been done, the influence of a second factor is determined. The influence of this factor is also determined graphically after the influence of the K-value has been eliminated. In the instance in question this was done by plotting the ordinate distances of the points from the curve in Fig. 12 against the corresponding values for the second factor. A point above the curve of averages indicates that the uptake was better than the average value, a point below it indicates the reverse.

Interactions between the factors are determined as far as possible. If the number of determinations is sufficient, they are divided into two or more groups of different K-values and the procedure is followed independently within these groups. In fact, it is very likely that the influence of the second factor on the K_2O-content of the potato tops (or something else) will be different depending on whether the K-value of the soil itself is high or low.

If the influence of a second factor on the K_2O-content of the tops is determined in this way, it can then be eliminated. The influence of the third factor is then determined in the same manner. When this has been determined, further corrections can be made. The adjusted K_2O-contents have been plotted against the K-value in Fig. 13. Moreover, it is possible to determine the influence of the second factor more accurately after the influence of the third factor *etc.* has been eliminated.

The relation between the lime status (here indicated as *lrb-value* *) and the deviations of the points from the average curve (Fig. 12) is demonstrated in Fig. 14 for a low K-value (< 20). The influence

* pH is not very suitable as an accurate indicator of differences in the lime status of calcareous soils because soils having varying contents of $CaCO_3$ show only slight differences in pH. On the other hand the $CaCO_3$ content is zero in the case of decalcified soils. Visser introduced the lrb-value (logarithm relative base content, lrb = log (S + + 20 × $CaCO_3$%)/T. The numerator is determined by measuring the degree of neutralization of added 0.1 *N* HCl; T = sorption in equilibrium with a surplus of $CaCO_3$) which can be determined in either case and allows the decalcified and calcareous soils to be considered on a single scale. In the case of soils with a neutral reaction but from which $CaCO_3$ is almost absent, lrb = 0. Decalcified soils give negative values whilst those containing $CaCO_3$ give positive values. In the case of a soil containing 1% $CaCO_3$ lrb = *ca* 0.2; whilst in the case of a soil containing 10% $CaCO_3$; lrb = *ca* 1.0.

of the third factor (content of particles less than 16 μ) has also been eliminated in this case.

The influence of the lime status is different. At a low K-value the K_2O-content appears to have been strongly affected by this factor. High K_2O contents have occurred on decalcified soils and low contents on moderately calcareous soils. At higher lrb-values there is an increase in K_2O content. Such a complex relation has frequently occurred in other series of potash experiments. There is little doubt that with increasing lrb values, decreasing K_2O contents are to be attributed to lime-potash antagonism. It is not so easy to give an explanation of an increase in K_2O content in the case of higher lrb values.

In the case of higher K-values, where potash no more acts as a primary factor the influence of the lime status was found to be less pronounced.

The influence of clay content is shown in Fig. 15. It was impossible to demonstrate an interaction for this factor and therefore all the determinations have been used. In this figure the influences of the K-value and the lrb-value have been eliminated. Soils with a higher clay content have yielded tops having lower K_2O contents than was the case with lighter soils.

There was no very distinct correlation between the K-values of the soils and relative yields in this series. The cause of this was probably associated with the fact that even the highest dressing (300 kg K_2O per ha) did not fully satisfy the requirements of the crop.

This difficulty in using relative yields as an index (this method has also been used by others) is clearly shown in the case of a river clay soil in an investigation by Ferrari [1]. He compared the use of relative yields with the use of absolute yields as tests of the K-value method (Figs. 16 and 17). It is clearly shown that a heavy dressing with 300 kg K_2O per ha did not suffice to satisfy the needs of the crop on this highly deficient soil. The use of relative yields (yield obtained without K-dressing as a percentage of the highest yield obtained with K-dressing on the trial plot)gives an erroneous picture of the real needs.

Attention may also be drawn to the possibilities of using mere visual observations of the crop. In one series simultaneous vigour scorings of the plots of all field trials were made on a scale of 1 10. These numbers have been reduced, in a similar way, to relative

vigour scorings by expressing the vigour score given to the crop not dressed with potash as a percentage of the score awarded to the crop receiving the maximum dressing on the same experimental field. These relative values show a good correlation which the K-values of the soil (Fig. 18); not differing very much from the correlation between the K-values and relative yields observed in the same experimental series.

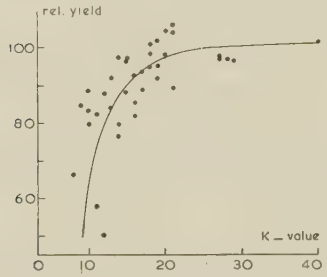


Fig. 16

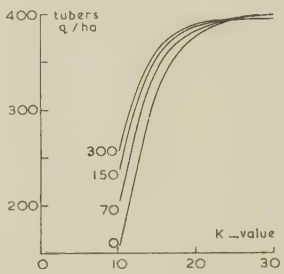


Fig. 17

Fig. 16. Relation between K-value of the soil and the relative yields of potatoes obtained in the absence of potash dressing and expressed as a percentage of the yields from the corresponding K(150) plots for river clay soil (according to Ferrari [1]).

Fig. 17. Relation between K-value of soil and yield of potatoes at 4 levels of potash application (kg K_2O per ha) for river clay soil (according to Ferrari [1]).

The investigations described above have provided the basis for the advisory scheme for potash dressings. The results permit the assessment of the relation between the influence on the reaction of the crop of a difference of one unit of the clay content scale and of one unit of the scale used for the indication of the lime status, to the scale of the K-value. With the aid of these relation the K-value is corrected to a value valid for soils with moderate clay and lime content.

Serious difficulties have still to be overcome. The investigations conducted in different years and regions do not always give similar results. The correlations with lime status and clay content especially do not agree in all cases. Nor do different crops behave similarly. River clay soils, showing less response to potash dressing owing to stronger fixation of potash by these soils, behave differently as compared with marine soils. Since only statistical relations have been

studied, these differences are not surprising. The real causal relations are still obscure.

For practical reasons it will be impossible to continue these investigations indefinitely. They are expensive and take much energy. From a scientific point of view the problem is, however, not yet solved. Differences between the results for series of experiments in different areas or different years require further explanation. A pot

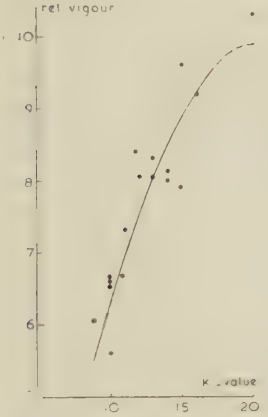


Fig. 18

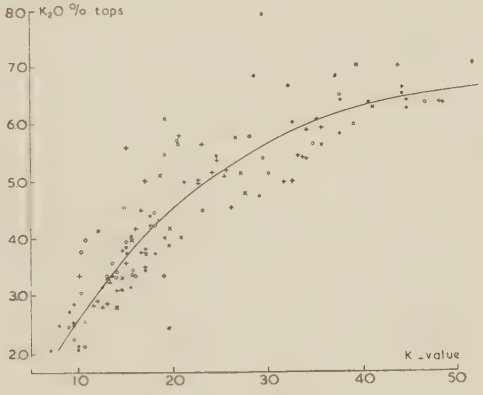


Fig. 19

Fig. 18. Relation between K-value of soil and relative vigour of the potato crop, after elimination of influence of variation in lime status ($CaCO_3$ = 1%) and content of particles < 16 μ (35%) for marine clay soil (1949).

Fig. 19. Relation between K-value of soil and K_2O content of potato tops in a pot experiment with clay soils of different origin (without corrections for interactions of the other factors).

experiment has therefore been carried out to investigate soils of different origin under comparable conditions. Oats, potatoes and beet were grown in succession on 123 samples of soil taken from different clay regions. These plants were used in view of their differing response to potash dressings and potash-lime antagonism. The crops were grown with (1 g K_2O per pot of 20 cm diametre) and without potash dressing and harvested at a rather young stage of development. The correlation between soil potash and the potash content of the potato plants is very good. This correlation is not affected by the origin of the soil (Fig. 19). However, one particular difference was noted between river and marine soils. Whilst the

potash dressing sufficed to satisfy the requirements of the plants completely in the case of the latter it did not do so in the case of the river soils. Investigations are continuing to elucidate the nature of the differences between these two soils.

It is clear that the results obtained in pot experiments are not applicable to field conditions. Correlations with lime status were, however, not different from those observed under field conditions.

3. Determination of the potash status of grassland from soil analyses

Until recently the K-value (HCl) method was also applied to grassland on clayey and peaty soils. Field trials similar to those described above for phosphate have been performed. The calibration of this value proved, however, to be impossible without con-

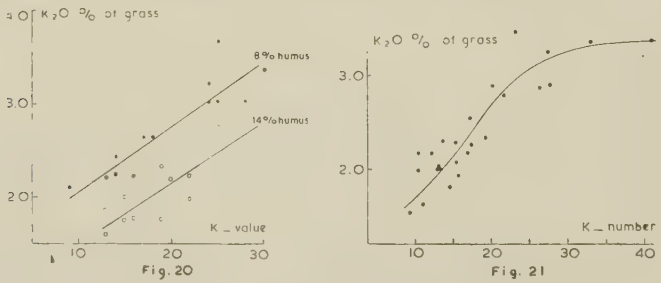


Fig. 20. Relation between K-value of soil and K_2O content of grass (without corrections for variations in N-content) at 2 different levels of humus content for river clay soils not dressed with potash (1948). The data obtained for soils containing 3.5–10.6% of humus have been adjusted to correspond to a humus content of 8%; those with 11.2–19.1% had been adjusted to correspond to a humus content of 14%.

Fig. 21. The same data as in Fig. 20 plotted against *K-number* instead of K-value).

sidering the influence of humus content. This is clearly shown by the relations observed between K-value of river clay soils having different humus contents and the K_2O content of the dry matter of the grass crops grown on these soils (Fig. 20).

The method used in the case of sandy soils * also proved to give

* According to this method an amount of soil containing 6.25 g of humus is extracted with 300 ml 0.1 *N* HCl. The ratio of potash released to humus content, multiplied by a certain factor, is determined. (This so-called *K-number* can also be derived from the K-value and the humus content by means of an empirical formula).

quite satisfactory results with this type of soil. The results used in Fig. 20 have been plotted against *K-number* in Fig. 21. It is clear from this that the method using K-number is preferable to that in which K-value is used since advice can be given without introducing a correction for humus content. In this case the influence of clay content was negligible, so that a test has been obtained which is very suitable for application to agricultural practice.

B. *Long-term experiments*

The picture obtained from field trials of one years duration is completed by experiments which are continued for several years. Such experiments were laid down on the main soil types throughout the country.

1. Phosphate experiments on arable land

These experiments are meant to give information about changes in phosphate status following the application of different phosphate dressings. The results are very interesting as it can be shown that the phosphate status on sandy and reclaimed moor soils tends to reach a state of equilibrium when there are constant yearly additions of phosphate (some examples in Fig. 22). It may be concluded that losses through leaching are much more important, in the case of ample dressings, than was hitherto considered to be the case. On clay soils, on the other hand, this phenomenon has not been observed. With ample phosphate dressings the P-citr values increase proportionally with time.

With moderate dressings changes in phosphate status take place relatively slowly, thereby indicating that the determination of phosphate status from soil analyses gives reliable indications which are valid for several years.

These long-term experiments may also give information about the value of soil analysis. It is difficult, however, to estimate the phosphate deficiency of any particular field as different crops with unequal requirements have been grown in different years. This difficulty has been partly overcome by evaluating the requirements of crops in the following way. The relation between relative yields (*i.e.* yields obtained in the absence of added phosphate expressed as percentages of the yields obtained with the highest phosphate dressing) for different crops grown in successive years is determined.

The results obtained for potato and rye on sandy soil are presented in Fig. 23. The responses of crops to added phosphate vary considerably in different years. Nevertheless a clear picture is obtained. It was also shown that the requirements of potatoes and beet are almost identical. The response of other crops has now been converted into that for beet and potatoes. In this way the average response to phosphate with these two crops in each field trial has

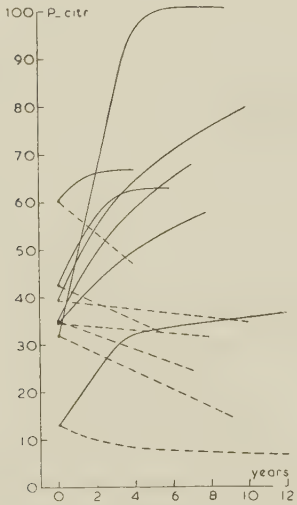


Fig. 22

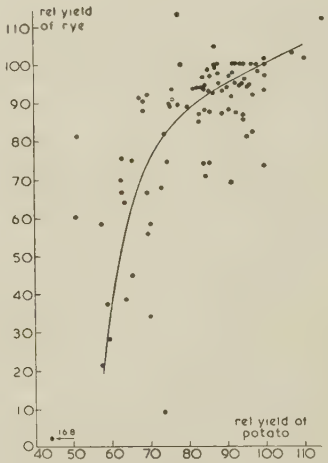


Fig. 23

Fig. 22. Variations in the phosphate status of arable land (sandy reclaimed moor) following heavy dressings (200 kg P_2O_5 per ha yearly) – continuous line curves, and in the absence of phosphate dressings – broken line curves; for a number of different experimental plots.

Fig. 23. Relation between relative yields of rye and potatoes obtained in successive years in the absence of phosphate dressing.

been determined. These mean relative yields have been plotted against the mean P-citr values obtained for the plots receiving no phosphate dressing. The result obtained on clay soils (Fig. 24) is rather illuminating.

Two experiments on loess soil gave results deviating from those on clay soil. Similarly it has been observed on grassland (p. 112, Fig. 8) that the P-citr value for this soil differs considerably from the value obtained in the case of other soils.

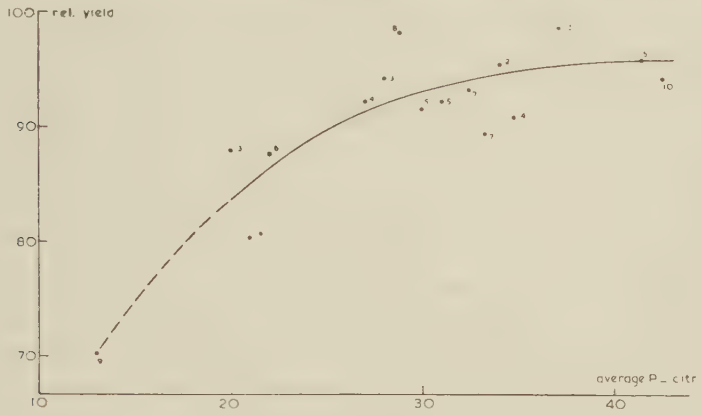


Fig. 24. Relation between P-citr values for clay soil and mean relative yield, in the absence of phosphate dressing, related to the response of beet and potato (found to be almost identical). The numbers associated with the various points indicate the number of the years of the experiments.

2. Potash experiments on arable land

It is less useful to analyse soil for potash than for phosphate. One of the reasons for this lies in the more difficult calibration of the analysis in relation to other factors affecting the availability of potash. Another reason concerns the greater variability of the results, especially on sandy and light clayey soils. Yearly fluctuations are much larger since the store of available potash is smaller and more readily changed by addition of fertilizer, absorption by plants and leaching.

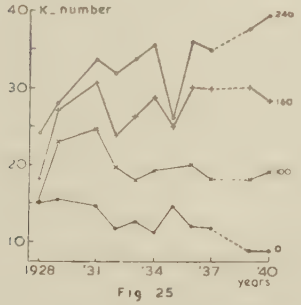


Fig 25

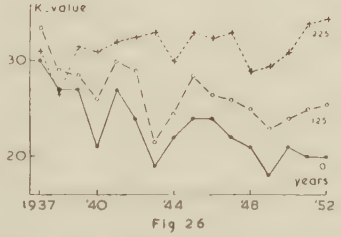


Fig 26

Fig. 25. Variation in the K-number over a trial-field (OO 51) on sandy soil, for different dressings of potash (kg K_2O per ha).

Fig. 26. Variations in the K-value over a trial field (ZHE 341) on marine clay soil.

Fig. 25 gives an example of the variations in the potash number on a sandy soil for different potash dressings under a rotation of potatoes and rye. Considerable fluctuations occurred. A certain level of equilibrium is reached after a relatively short time. Excess potash is leached down into the subsoil. This is especially the case if the potash status has been raised to a high level by heavy dressings, and potash dressings are then subsequently omitted.

Another example concerning a clay soil containing 50% of particles $< 16\ \mu$ (Fig. 26) shows that in this case also fluctuations occur and that no considerable differences in soil potash level arise, despite large differences in the amounts of potash applied. The value of the test is restricted by this drawback. Therefore, advice on the basis of the potash test has a more limited value. Only for the first year can exact advice be given. The advice for the following years will be vague. The usefulness of the test rests primarily on the fact that it permits a critical comparison of the potash status of different plots of the same farm and comparisons between farms and regions.

The above examples may suffice to give an impression of the work done in this field in the Netherlands. For more extensive information the reader is referred to previous papers on the subject in Dutch [1] [3] [4] [5] [6] [7] [8] [9] [10]. Summaries and charts, with information in English, have been included in most cases.

SUMMARY

The practical value of soil analyses depends to a large extent on the accuracy of the calibration of the tests under field conditions. Examples are given to illustrate the procedure followed in work performed in the Netherlands along these lines.

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SOME OBSERVATIONS ON THE CORRELATED INFLUENCE OF FERTILIZERS ON PEANUT YIELDS AND VEGETATIVE DEVELOPMENT OF THE PLANTS

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INTRODUCTION

In experiments to test the effect of fertilizers on peanut yields it is difficult to get consistent responses, and this difficulty has been the subject of much discussion. York *et al.* [6], in the symposium on "The Peanut, The Unpredictable Legume", write, "A review of the peanut fertility research.. reveals a multitude of inconsistent, and apparently conflicting data".

The vegetative development of the peanut plant has also been widely studied, especially by workers in French Afrika [3]. But it seems that little attention has been paid, to the relation between the use of fertilizers, the vegetative development, and the yields. It has been shown that vegetative development, (expressed as hay yield) increases along with increased pod yields, as a result of applications of nitrogen fertilizer [1]. It is claimed, on the basis of other data [2], however, that nitrogen fertilizer tends to increase top growth of the plant without increasing pod yield. Greenwood [4] obtained a positive response to nitrogen with peanut seedlings grown in containers up to the age of 30 days, but not with plants under field conditions.

The purpose of the work reported in the present paper was to determine if there is a correlation between pod yield and the vegetative development of the peanut plant as influenced by fertilizers.

METHODS

Because fertilizer experiments with peanuts under field conditions frequently fail, the experiment was carried out in containers. They were about 45 cm high and 60 cm wide, and were sunk into the ground; they were filled

with red sandy loam that had been taken from the exposed subsoil on an eroded slope with no cover of vegetation. The soil was thoroughly mixed before use.

Three fertilizers were applied at two rates, as shown in the schedule below:

Fertilizer	Designation	g per container	Nutrient equivalent	kg per ha
Ammonium sulphate .	N	27	N	150
Triple superphosphate .	P_1	4.1	P_2O_5	50
,, ,,	P_2	12.3	P_2O_5	150
Potassium chloride . .	K_1	2.1	K_2O	30
,, ,, . .	K_2	10.5	K_2O	150

The fertilizers were applied in the following combinations:

1. Control, unfertilized.
2. N
3. K_1
4. K_2
5. P_1
6. P_2
7. N + K_1
8. N + K_2
9. N + P_1
10. N + P_2
11. K_1 + P_1
12. K_1 + P_2
13. K_2 + P_1
14. K_2 + P_2
15. N + K_1 + P_1
16. N + K_1 + P_2
17. N + K_2 + P_1
18. N + K_2 + P_2

Each formula was applied in 5 replications, in a completely randomized block design. Three seeds of "Virginia Bunch" peanuts were sown in the center of each container. After germination the seedlings were thinned out to two. The sowing was done on 4 May, 1953, and the plants were harvested on 5 October, 1953. During the growing period the plants were irrigated once a week, each container receiving a total of 184 litres of water (about the equivalent of 520 mm of rainfall). After harvesting, the plants were air dried, and weighed with the pods still on them; then the pods were separated from the haulms and weighed separately. Hay weights were then calcualted by subtracting the pod yield of each plant from its total weight. The data on root development were obtained by measuring the length of all roots branching off the tap root, plus that of the tap root itself. Second-degree root branches were discarded. Nodule counts were made on a total root length of 20 cm from each container, consisting of bits of roots of the first branching (excluding the tap root).

RESULTS

Table I shows the influence of the various treatments on yields and vegetative development. Analysis of variance gave very significant results for all four kinds of data.

TABLE I

The influence of fertilizers on yields and vegetative development of the peanut plant *)

Treatment	Pod yield (g per container)	Hay yield (g per container)	Root length (cm per container)	Nodules (per 20 cm of root) **)
Control	97.9	106.9	470	93
N	40.1	49.0	234	7
K_1	109.8	108.9	427	102
K_2	65.0	81.3	415	107
P_1	118.9	145.9	294	141
P_2	126.2	146.5	318	135
N + K_1	83.6	82.2	219	41
N + K_2	58.7	56.3	177	12
N + P_1	141.8	167.2	230	65
N + P_2	111.6	138.6	154	48
K_1 + P_1	107.1	122.7	409	122
K_1 + P_2	132.5	145.9	322	120
K_2 + P_1	136.6	151.6	379	121
K_2 + P_2	107.6	116.5	330	112
N + K_1 + P_1	123.6	142.6	122	55
N + K_1 + P_2	147.7	197.3	206	40
N + K_2 + P_1	140.8	176.2	197	63
N + K_2 + P_2	138.7	178.8	238	33
Significant difference	40.9	42.9	132	37
Very significant difference	54.8	56.9	175	48
F value of analysis of variance	4.65++	8.67++	4.80++	8.53++

* Means of 5 replications.

** The data on nodule numbers will also been presented in a following paper, "The influence of fertilizers on nodulation in peanuts".

It can be seen from Table I, even without a statistical analysis, that there is a fair correlation between hay yields and pod yields. It is also obvious that all the formulae in which P was included, increased yields, as compared to the control, whereas all formulae which contained N but no P, decreased yields. Correlation coefficients are given in Table II.

TABLE II

Correlation coefficients between yield data and vegetative development data in peanuts

Correlated data	Correlation coefficient
Pod yield × hay yield	+ 0.824++
Pod yield × root length	— 0.094-
Pod yield × nodule number	+ 0.292++
Hay yield × nodule number	+ 0.205+
Hay yield × root length	— 0.156-
Nodule number × root length	— 0.219+
Minimum points: at $P = 0.05$ — 0.205; at $P = 0.01$ — 0.267.	

\- = not significant; + = significant. ++ = very significant.

The calculated correlation coefficients prove that the positive correlation between hay yield and pod yield is very significant, and so, also is the positive correlation between pod yield and nodulation. A positive correlation between hay yield and nodulation and a negative correlation between nodulation and root length are just significant. Table III presents the results of factorial analysis of the data, of which the averages were given in Table I.

TABLE III

F values of detailed analyses of the effect of fertilizers on plant development and yield of peanuts

	Pod yield	Hay yield	Root length	Nodulation
A. Main effects				
N	0.06^{-} (—)	0.93^{-} (+)	64.00^{++}(—)	149.29^{++}(—)
P	25.77^{++}(+)	4.46^{+} (+)	3.06^{-} (—)	9.98^{++}(+)
K	1.05^{-} (+)	0.93^{-} (+)	0	0.44^{-} (—)
B. Interactions				
N × P	4.40^{+}	9.49^{++}	1.82^{-}	0.58^{-}
P × K	2.31^{-}	2.78^{+}	0.71^{-}	1.25^{-}
N × K	1.30^{-}	2.35^{-}	0.46^{-}	0.39^{-}
N × P × K	0.91^{-}	0.74^{-}	1.41^{-}	0.40^{-}
C. Levels				
P_2 against P_1	0	0.11^{-} (—)	0.16^{-} (—)	2.85^{-} (—)
D. Factorial				
N without P	6.49^{+} (—)	8.68^{++}(—)	35.54^{++}(—)	55.25^{++}(—)
N with P	2.26^{-} (+)	10.67^{++}(+)	31.50^{++}(—)	94.26^{++}(—)
P without N	4.48^{+} (+)	6.68^{++}(+)	4.62^{+} (—)	3.52^{+} (+)
P with N	25.66^{++}(+)	47.44^{++}(+)	0.26^{-} (—)	7.08^{++}(+)

(+) = positive effect
(—) = negative effect

$^{-}$ = not significant
$^{+}$ = significant
$^{++}$ = very significant.

Table III demonstrates the opposed effect of N and P on peanut yields and development, especially on nodulation, and the change in the effect of N due to its interaction with P in some cases. It also shows that root length invariably decreases, wherever fertilizers have a significant influence on the plants.

DISCUSSION

The most conspicuous fact shown by the data is the high positive correlation between pod yield and vegetative development, as expressed in hay yield. It is also obvious from Table III that all factors which significantly increased hay yields also increased pod yields.

Another important result is the evidence of correlation between nodulation and yields. These correlations, along with the interactions among fertilizers, call for a detailed examination of the effects of the fertilizers.

The effect of N

The effect of N is strongly negative with regard to all aspects of the vegetative development, as well as to pod yield, when applied by itself. In combination with P, however, its effect is very significantly positive for hay yield, and sub-significantly positive for pod yield, while it remains very significantly negative for root length and for nodulation (Plate I). This may be interpreted to mean that while N fertilizer inhibits nodulation, at the same time it substitutes mineral N for the nitrogenous contribution of the nodule bacteria towards plant nutrition. The fact that mineral N, as a substitute for bacterial contribution, is more effective for hay yield than for pod yield suggests that nitrogenous compounds produced by the nodule bacteria may be better suited for the construction of the seed proteins than for the proteins of the green tissue. It must be observed, however, that in this experiment, contrary to Batten's results [2], every increase in hay yield was accompanied by an increase in pod yield. The increase in pod yield due to N alone, which is sometimes reported [5], may be due to the interaction between N and P. For, while N in itself has an inhibiting effect on plant development and on yields, it may, where P is available in the soil, interact with it, and thus increase yields.

The effect of P

The most remarkable aspect of the effect of P is that besides its positive effect on pod yield, hay yield, and nodulation, it is also able to change the effect of N on yields from negative to positive. The nature of this interaction can only be guessed at: it may be that a plant is better able to utilize mineral N if P is available, while if P is lacking the plant must depend on nodule-produced N compounds.

Another interesting aspect of the effect of P is that the high rate of application had no advantage over the low level, which was only a third as great.

PLATE I. Influence of nitrogen on nodulation

The effect of K

No significant effect of K was noted in this experiment, except for a slight interaction of K with P affecting the hay yield.

SUMMARY

The effect of P, K, and N fertilizers on vegetative development and on yields of peanuts was tested with "Virginia Bunch" peanuts, grown on sandy-loam subsoil material, in containers.

P and K fertilizers were applied at two rates each, and N fertilizer, at one rate.

The experiment was factorial. The resulting data of pod yields, hay yields, total length of roots, and rate of nodulation, were tested statistically, to determine if there are correlations between various expressions of vegetative development and pod yield, and if there are interactions among the various fertilizers.

1. Positive correlations were found between pod yield and hay yield, between pod yield and nodulation, and between hay yield and nodulation.

2. A negative correlation was found between root development and nodulation.

3. Significant interaction was found between P and N; the effect of N on yields was negative, when applied alone, but was positive when combined with P.

4. The high rate of P application showed no advantage over the rate only one third as great.

5. No significant effect of K fertilizer was obtained, though there was a slight interaction of K and P on hay yield.

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BIOLOGICAL CONTROL OF A SOIL-BORNE PYTHIUM INFECTION BY SEED INOCULATION

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It is not always possible to infect a soil with a disease-producing organism by introducing the pathogen into unsterilized soil. If, after a short period, seeds of a natural host are sown in such an artificially diseased soil, healthy plants may develop. After sterilization of the soil, however, introduction of the pathogen will usually cause considerable damage to the seedlings (Sanford and Broadfoot [9], Vasudeva and Roy [13]). This has been attributed to removal of the normal soil microflora which antagonized the survival and spread of the pathogen in unsterilized soil.

Observations of this kind led to attempts to control diseases, especially soil- and seed-borne diseases, by the introduction of saprophytes, known to antagonize the particular pathogen *in vitro*, into the soil either by direct inoculation or by altering the soil conditions to favour growth of antagonistic saprophytes already present in the soil.

One common soil inhabitant in particular, *Trichoderma viride*, is antagonistic to a range of pathogens both *in vitro* and under soil conditions. Weindling [14 15] and Weindling and Fawcett [16] showed that introduction of spores of *T. viride* into soil infected with *Rhizoctonia solani*, a fungus causing damping-off of *Citrus* seedlings, effected good control of the disease, especially in acid soils. The fungus was shown to produce, in culture media, an antibiotic, gliotoxin, which is stable only in acid conditions. If control of disease were due to production of gliotoxin in the soil this would explain the better control of disease under acid conditions. *T. viride* has also been shown to control or reduce diseases caused by *Pythium debaryanum* (Gregory, Allen, Riker and Peterson [4]), *Sclerotium*

rolfsii (Morton[7]), *Fusarium lini* (Timonin[10]), *Fusarium culmorum* (Johnston and Greaney[5]), *Helminthosporium sativum* (Anwar[1]), *Armillaria mellea* (Bliss[2]) and *Fomes annosus* (Rishbeth[8]).

A study has here been made of the effect of dusting seeds with spores of *T. viride* and other soil saprophytes on the course of a soil-borne disease caused by *Pythium* sp. This fungus is not specific to one host or even to related plants; it was found to be pathogenic to a wide range of seedlings including mustard, cabbage, letttuce, lucerne, flax, wheat, rye and oats. The diseased plants have brown and stunted root systems and the shoots of the dicotyledonous seedlings bear dark necrotic areas.

METHODS

Cultures

Pythium sp. was isolated from an infertile patch of soil in East Lothian and proved to be pathogenic to a wide range of seedlings.

Trichoderma viride Pers. ex Fries. Strain No. 211 which produces high yields of gliotoxin in culture media was isolated from a greenhouse soil. Strain No. 941 produces viridin in culture media. Strain No. 363 which was isolated from a Scots Pine root does not, in our experience, produce any antibiotic in culture; this is referred to in the text as an inactive strain.

Penicillium nigricans Bainier (= *P. janczewskii* Zal.) (No. 250) produces griseofulvin; it was first isolated from Wareham Heath soil.

P. frequentans Westl. (No. 812), isolated from Wareham Heath soil, produces frequentin.

P. godlewskii Zal. (No. 869) also isolated from Wareham Heath soil, caused stunting of germ tubes of *Botrytis allii* in a serial dilution test for antifungal activity.

Seed

White mustard was used throughout this work as it is very susceptible to attack by the pathogen.

Soil

John Innes potting compost No. 2 (pH 5.2–6.5) was used.

Inoculation of soil

Soil supplemented with 5% (w/w.) wheatmeal was autoclaved (15 lb/in.^{2} for 45 minutes) and then inoculated with mycelial fragments from a culture of *Pythium* sp. grown on Czapek-Dox agar. After 7 days' incubation at

25°C, 2% (w/w) of the soil/wheatmeal inoculum was thoroughly mixed with unsterilized soil and dispensed in 200g portions into beakers. This soil was incubated for a further 4 days and 25 seeds of white mustard were sown in each beaker and the seeds left to germinate in a greenhouse. Three replicate beakers were set up for each treatment.

Inoculation of seed

The seeds were lightly coated with spores of the potential antagonists by shaking them in bottles in which sporing agar cultures of the fungi were growing.

Assessment of disease

Counts of the numbers of seeds which germinated and numbers of seedlings bearing typical black lesions on the cotyledons and hypocotyls were made after 3, 7, 10, 14 and 17 days. After 21 days the numbers of seedlings which had survived and the fresh weights of the shoots were recorded. The numbers of seeds which had germinated and of healthy seedlings which survived were expressed as percentages of the total number sown. The fresh weight of the shoots was expressed as a percentage of that of untreated plants. Where figures are given in the tables these are based on the total weight of the seedlings which survived in each treatment rather than on mean fresh weight/plant. This was considered a more accurate measure of estimating the effect of the disease for in those cases where disease was most severe the few plants which survived grew vigorously due to lack of competition compared with the untreated control seedlings in the more crowded beakers and hence any figures based on mean fresh weight/plant would be misleading.

RESULTS

Effect of size of inoculum of Pythium *sp. on disease symptoms*

The proportions of soil/wheatmeal inoculum of *Pythium* sp.mixed with untreated soil was varied as follows: 1%, 2%, 5% and 10%. The results are presented in Table I.

TABLE I

Effect of size of inoculum of *Pythium* sp. on disease symptoms			
Inoculum	% Germination	% Healthy plants surviving after 21 days	Weight of plants expressed as % control
None	95	92	100
1%	96	75	83
2%	78	44	55
5%	77	24	50
10%	68	14	37

It can be seen that the disease has a 3-fold effect on the seedlings, of white mustard. Germination was slightly reduced, the number of healthy plants surviving after 3 weeks was greatly reduced and the total weight of the shoots was much lower than that of plants not exposed to the disease. The diseased plants were stunted and bore black, depressed pin-point lesions on the cotyledons and long dark necrotic streaks on the hypocotyls.

Effect of dusting seeds with fungal spores on production of disease symptoms

Seeds of mustard were dusted with spores of *T. viride* (No. 211), *P. nigricans*, *P. frequentans* and *P. godleswkii*. The effect of these fungi on the course of the disease is shown in Table II.

TABLE II

Effect of dusting seeds with fungal spores on production of disease symptoms of *Pythium* sp.

Seed dusted with	% Germination		% Healthy plants surviving after 21 days		Weight of plants expressed as % control	
	Untreated soil	Infected soil	Untreated soil	Infected soil	Untreated soil	Infected soil
Not dusted	91	71	87	12	100	63
Trichoderma viride	92	76	88	36	101	81
Penicillium nigricans	91	60	89	10	103	59
P. frequentans	89	81	84	29	102	84
P. godlewskii	92	80	88	55	106	90

All the fungi tested, with the exception of *P. nigricans*, afforded some protection to the seedlings sown in diseased soil. Germination was higher, the number of healthy seedlings surviving after 3 weeks was greater and the shoots were heavier.

Antagonism of three strains of T. viride *to a disease produced by* Pythium *sp.*

Mustard seeds were dusted with strains of *T. viride* know to produce gliotoxin or viridin, and with the inactive strain.

All strains, irrespective of their capacity to produce antibiotics, controlled the disease to some extent (Table III). The gliotoxin-

producing strain was most effective against the pathogen and the inactive strain was least effective.

TABLE III

Antagonism of three strains of *T. viride* to a disease produced by *Pythium* sp.

Strain of *T. viride* used for seed inoculation	% Germination		% Healthy plants surviving after 21 days		Weight of plants expressed as % control	
	Untreated soil	Infected soil	Untreated soil	Infected soil	Untreated soil	Infected soil
None	97	79	78	26	100	71
Gliotoxin-producing	92	92	77	72	98	83
Viridin-producing	95	83	76	61	100	78
Inactive	85	82	76	43	91	71

With heavy inocula of the antibiotic-producing strains of *Trichoderma*, the seedlings, from both infected and untreated soil, developed chlorotic patches on the cotyledons; this was not observed on seedlings derived from seed inoculated with the inactive strain.

Effect of soil pH on control of disease produced by Pythium *sp.*

The natural pH of the soil (pH 6.0) was altered by acidifying with dilute sulphuric acid to give a soil reaction of pH 3.5 or by addition of calcium hydroxide to give an alkaline soil (pH 7.8). Mustard seeds, either untreated or dusted with spores of the gliotoxin-producing strain of *T. viride*, were sown in the soil.

In this experiment, the beneficial effect of dusting the seeds with *T. viride* spores was not as marked as formerly (Table IV). In acid

TABLE IV

Effect of soil pH on control of disease produced by *Pythium* sp.

pH of soil	Treatment of seed	% Germination		% Healthy plants surviving after 21 days		Weight of plants expressed as % control	
		Untreated soil	Infected soil	Untreated soil	Infected soil	Untreated soil	Infected soil
6.0	None	95	85	89	43	100	87
6.0	Dusted with *T. viride*	92	88	87	49	109	77
3.5	None	96	93	91	85	64	81
3.5	Dusted with *T. viride*	97	92	94	81	63	83
7.8	None	96	85	96	69	94	106
7.8	Dusted with *T. viride*	95	93	95	80	100	107

soil plant growth was reduced but the disease was much less severe. Dusting the seeds with *Trichoderma* spores gave no further control of the disease.

Growth of the seedlings improved upon addition of calcium hydroxide to the soil and this treatment also reduced the spread of the disease, although not to the same extent as the acid treatment. Dusting the seeds with spores of *T. viride* afforded greater protection to the seedlings in this soil.

DISCUSSION

The species of *Pythium* which was isolated from a Scottish soil causes considerable damage to seedlings of many varieties of plants. Disease symptoms can be controlled to some extent in the case of white mustard by dusting the seeds with spores of some common soil saprophytes. All the fungi tested for ability to control this disease, with the exception of *Penicillium nigricans*, gave some protection to seedlings of mustard grown in diseased soil. These fungi, with the exception of one strain of *Trichoderma viride* (see below), produce antifungal antibiotics on synthetic media. *P. nigricans*, which was ineffective as an antagonist, produces the antifungal antibiotic griseofulvin. This antibiotic is not inhibitory to species of *Pythium* (Brian[3]) and accordingly even if the antibiotic were produced in soil this in itself would not be expected to confer antagonistic properties on *P. nigricans* in relation to *Pythium*.

Of the three strains of *Trichoderma viride* which were tested, the most effective in controlling the disease was the gliotoxin-producing strain. If control is effected by production of antibiotics, it might be expected, on the basis of antifungal activity, that the strain producing viridin, a more highly antifungal antibiotic, would give the best control. The conditions under which viridin is produced, however, are much more limited than those suitable for gliotoxin production, especially in soil, where gliotoxin has been shown to be produced under natural conditions (Wright[17]). Moreover, viridin is far less stable in aqueous solution than gliotoxin. The inactive strain of *T. viride* is not as effective as antibiotic-producing strains but does afford some protection to the seedlings. It would seem, therefore, that ability to control the disease may not be entirely dependent upon capacity to produce an antibiotic.

Weindling found that control of *Rhizoctonia solani* by inoculation of the soil with *T. viride* was best under acid conditions. This was explained in terms of gliotoxin production by *T. viride* for the antibiotic is stable in acid conditions but loses activity in media of high pH. In these experiments, however, although the disease caused by *Pythium* sp. was less severe when the soil was acidified, no further control was obtained by inoculating the seeds with spores of *T. viride*. The severity of the disease was also reduced upon addition of calcium hydroxide to the soil and, in this case, it was controlled still further by dusting the seeds with *T. viride* spores. These results are apparently contradictory to those of Weindling. This experiment was repeated several times and similar results were obtained each time.

It is, however, possible, to suggest an explanation of these apparently anomalous results. If seeds were heavily coated with spores of the gliotoxin-producing strain of *T. viride*, damaged seedlings developed. The cotyledons were wholly or partially chlorotic; a similar effect was produced when seeds were germinated in solutions of gliotoxin.

Later work, which will be described in another publication, has confirmed that high concentrations of gliotoxin may be formed in and around seed coats which have been inoculated with *T. viride*. In acid soils, any gliotoxin produced would be fairly stable and concentrations of the antibiotic accumulating around the seed might well be phytotoxic. Damaged seedlings so produced might be expected to be more susceptible to attack by *Pythium* sp. and this would tend to minimise the protective effect of seed inoculated with *T. viride*.

In soil treated with calcium hydroxide growth of the seedlings was more vigorous and they were less susceptible to attack by *Pythium* sp. than in untreated soil. Inoculation of the seed with *T. viride* gave further protection to the seedlings. In this case one can envisage that enough gliotoxin may have been produced around the acid seed coat to give some protection to the seedlings while the high soil pH prevented accumulation of a concentration sufficient to cause damage.

Thus the presence of certain soil saprophytes on the seed coat affords some protection to the seedlings of white mustard growing in soil infected with a soil-borne pathogen, *Pythium* sp. Protection of alfalfa seedlings from attack by *Pythium debaryanum* has also been

obtained by incorporating *T. viride* into a pellet-mixture surrounding the seed (Gregory, Allen, Riker and Peterson [4]). It would be expected that such inoculation of seeds would be more effective against seed-borne diseases for here the antagonist would come into closer contact with the pathogen. Tveit and Wood [12] have reported very good control of *Fusarium* blight of oat seedlings by inoculation of the seed or the soil with isolates of *Chaetomium*, some species of which produce antibiotics in culture media. Control of *Fusarium* blight by this method was as good as that given by mercurial dusts.

In those cases where, under natural conditions, diseases introduced into soil artificially or on contaminated seeds do not spread it is possible that seedlings are protected by the antibiotic-producing organisms present in the soil or indigenous on the seeds themselves (Ledingham, Sallans and Simmonds [6]), Tveit and Moore [11]). Prerequisites for such control are soil conditions favourable to antibiotic production and a suitable microflora. In soils where a disease is established and not amenable to control by other methods, alteration of the soil conditions to favour growth of antagonistic saprophytes, the presence of which could be ensured by inoculating the seed, is a possible control measure.

SUMMARY

1. A three-fold effect was produced on white mustard seedlings grown in soil infected with *Pythium* sp. Seed germination, the number of healthy plants which survived and the fresh weight of the shoots were reduced.

2. Disease symptoms were controlled to some extent by dusting the seeds with spores of some common soil saprophytes including *Trichoderma viride*, *Penicillium nigricans*, *P. frequentans* and *P. godlewskii*.

3. Of three strains of *Trichoderma viride* which were tested for antagonism to *Pythium* sp., a gliotoxin-producing strain was more effective in controlling the disease than a viridin-producing strain and an antibiotically inactive strain gave least protection to the seedlings.

4. The disease symptoms were less severe in soil treated with acid or calcium hydroxide. Inoculation of the seeds with *T. viride* gave further control of the disease in soil treated with calcium hydroxide but not in acidified soils. These results are discussed in relation to the production of gliotoxin by *T. viride*.

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PLANT NUTRITION ON FLY-ASH

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I. INTRODUCTION

The term "Fly-ash" is used to describe an industrial waste material which is produced after the combustion of pulverized coal. Modern power stations produce large quantities of this waste material and the satisfactory disposal of it may present a problem. In some cases it is deposited on low-lying meadows in the form of a low spoil heap or used as a filler for valleys and sand pits. When tipping operations are completed it is anticipated that attempts will be made to cover the surface of the ash with some form of plant cover. The field experiments of Rees and Skelding [12] on the summit of a spoil heap at Hams Hall power station, Warwickshire, indicated that a grass ley could be established if the surface of the ash was first covered with a layer of sewage sludge or sludge soil mixtures. There were no drought problems although the ash deposit was about forty feet in height. A problem which may arise however concerns the effect of the ash particles on plants roots. This might occur in time when soil and ash became intermixed if the practices of rotational farming were employed on such areas. A knowledge of the plant ash nutrition interrelationships would appear important in this respect, such information would be valuable also in cases where a soil or sludge covering was unavailable and a direct seeding of the ash was the only suitable alternative. The natural plant colonization of freshly deposited Hams Hall ash differs markedly from early successional stages which characterize the Black Country waste areas (Rees [8]). The power station ash becomes covered with the

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chenopodiaceous plant *Atriplex hastata* var. *deltoidea* which often forms an almost pure stand (Plate Ia) when other weeds and rough grasses are excluded and it was the object of the present investigation to examine ash properties responsible for the exclusion of common weeds and rough grasses which figure prominently in the early plant succession on many other industrial waste materials. It was decided therefore to include *Atriplex* in the experiments using it as a test plant for comparison with various crop plants which were used to examine the plant nutrition potential of the ash.

II. THE COMPOSITION OF THE ASH

1. *Physical properties*

The ash is composed of very fine particles which are easily wind borne and a mechanical analysis of it carried out by hydrometer method and expressed on the Atterberg scale is given in Table I.

TABLE I

Mechanical analysis of the ash		
Constituent	Particle size (mm)	Value (per cent)
Coarse sand.	2—0.2	19·78
Fine sand	0·2—0·02	54·00
Silt	0·02—0·002	22·00
Clay	< 0·002	3·10
Soluble matter . . .		1·12

Table I shows that much of the ash is composed of particles of less than 200 microns and replicate measurements indicate that its physical composition is very uniform. The conditions for plant growth might be expected to be somewhat similar to those of a sand culture medium. Its water holding capacity is about 100 per cent and there is no evidence of moisture impedance under field conditions.

2. *Chemical analysis*

A detailed chemical analysis has been obtained from a number of sources. An approximate analysis of the major constituents, kindly supplied by the Central Electricity Authority, is given in Table II. The chief feature of interest woud appear to be the relative high concentrations of oxides of alkaline and alkaline earth metals.

TABLE II

Proximate analysis of the ash			
Constituent	Percentage	Constituent	Percentage
Silica	43·40—51·8	Oxides of potash and sodium	2·60—3·13
Alumina	15·50—29·34	Sulphuric anhydride	1·34—1·88
Ferric oxide	8·25—0·48	Chlorides	0·16—0·30
Ferrous oxide	5·96	Phosphorus pentoxide	0·11—0.11
Calcium oxide	6·42—6·82	Combined carbon-di-oxide	0·06—1·85
Magnesium oxide	1·21—1·60	Combined water	0·13—0·58
Manganous oxide	N.D.—0.42	Carbon	2·12—3·34
Nickel oxide	N.D.—0·01	Free moisture	N.D.—0·27
Titanium oxide	0·60—0·92	Undetermined matter	0·21—0·34

These figures represent the chemical composition of the material as produced in the furnaces. Much of the soluble material will be removed during its prolonged contact with water in the settling ponds in which it may remain for many months before transport to the deposition area. Nevertheless the ash still contains much basic material and the value of the field pH is often greater than 8·5.

The data presented in Tables III, IV and V represent analyses made on a number of randomized samples collected from the ash tip. Table III summarizes the total trace element content as determined by spectrograph. In contrast the values given in Table IV represent what is interpreted by some investigators as the so-called "available" amounts and it will be observed that only about a half listed in Table III can be considered available to plants, and then in only a proportion of their total content in the ash. However, a comparison of the ash and soil available metals (columns 2 and 3 respectively of Table IV) suggest that there is a possibility that Co, Cr, Cu, Fe, Ni, V and Zn may be present in quantities harmful to plants. Evidence will be produced later which suggests that as far as the present observations go these metals are not present in toxic quantities. A number of other conventional "soil nutrient" techniques were also employed to enlarge the general picture of ash nutrient properties, and some of these are summarized in Table V. It would appear that the Morgan's extract may give a truer indication of metal available than, for example, the soil solution, since the amount of aluminium determined by the latter method is much less than amounts present in normal soils. Evidence will be produced later however of aluminium toxicity symptoms exerted by the ash.

The analytical data obtained indicates that the ash may contain

TABLE III

Total trace element content of the ash.
Amounts as parts per million oven dry matter (mean of 4 determinations)

Element	ppm	Element	ppm
Ag	1	Mo	6·50
Be	20	Ni	200·0
Bi	30	Pb	500
Cd	200	Rb	107·5
Co	30	Sc	16·25
Cr	200	Sn	5
Cu	200	Sr	500
Ga	21·25	Ti	3500
Go	17·50	V	300
La	55·00	Y	200
Li	212·50	Zn	750
Mn	3000	Zr	200

TABLE IV

2·5 per cent acetic acid-soluble soil and ash trace elements.
Amounts as parts per million oven dry matter

Element	Ash	Soil	Element	Ash	Soil
Ag	1	1	Ni	60·00	2·70
Co	8·45	1·58	Pb	10·00	10·00
Cr	14·25	1·70	Sn	10·00	10·00
Cu	13·00	3·50	Ti	15·25	10·00
Fe	1774·00	N.D.	V	5·97	1·30
Mo	0·80	0·30	Zn	212·75	168·00

TABLE V

The concentration (ppm) of certain important plant nutrients in the ash as determined by various methods *

Nutrient	Total	In water extract	In CO_2 water extract	In displaced solution	In normal ammonium acetate extract	In 2.5% acetic aicd extract	In Morgan's extract	Method
Nitrogen	490	—	—	—	—	—	—	Macro-Kjeldahl
Phosphate	—	60·0	65·0	—	26.0	625.0	1690·0	Wolf's
Calcium	1770	—	—	—	—	—	—	Distillation
	—	1060·0	—	707.5	—	—	—	Flame photometer
Potassium	—	178·0	—	134·5	—	—	—	,,
Magnesium	—	—	—	231·0	—	—	—	Lindner's
Iron	—	N.D. †	1	N.D.	1	1066	1820	Thiocyanate'
Manganese	—	N.D.	32·0	1	9.55	233·0	264·0	Periodate
Aluminium	—	6.6	2.2	1	N.D.	143.5	433·2	Aluminon
Cobalt	—	N.D.	N.D.	N.D.	N.D.	5·0	2.0	Thiocyanate
Nickel	—	N.D.	N.D.	N.D.	N.D.	42.0	16·4	Dimethylglyoxime
Sodium	—	218·0	—	386·0	—	—	—	Flame photometer
Lithium	—	17.45	—	13.5	—	—	—	Flame photometer

* Amounts expressed on oven dry ash basis.

† N.D. = not detected.

a sufficiency of all the essential plant nutrients with the exception of nitrogen. A number of metal ions appear to be present in toxic proportions.

III. EXPERIMENTAL

As this paper is somewhat in the nature of a pioneer investigation in the field of plant nutrition, each successive step in the project depends to a certain extent on the observations of previous experiments. It seemed valid and appropriate therefore to describe techniques employed with the individual experiments in favour of adopting the more conventional method which separates "materials and methods" from "experiments".

1. *Preliminary observations on the nutritional status of plants grown on ash*

Early observations (Rees and Skelding[12]) on the growth of a grass ley mixture on the bare ash containing an N.P.K.-fertilizer showed that the early yields of grass were practically negligible compared with controls. These results suggested that the ash may contain substances which retard ley development and in June 1954 a pilot experiment was conducted to compare the growth of a crop and garden plant, barley and spinach respectively, with that of the successful weed *Atriplex*. Spinach was chosen because of its close taxonomic connection with *Atriplex* while barley is known to be sensitive to a variety of soil disorders.

a) Culture procedure. Seeds of *Atriplex*, collected in the autumn of 1953 from a single plant growing on the ash tip, were sufficient to supply the needs of this investigation. Barley and spinach seeds were obtained locally. One week old seedlings which had been reared in acid washed sand were transplanted to bitumen-painted pots (Hewitt[6]) containing three different media, garden soil with fertilizer, ash with fertilizer and ash alone. The three treatments were employed in quintuplicate. The pots were kept in a greenhouse at 20°C ± 5°. Barley and spinach plants were harvested for analysis after thirty days but the slower growing *Atriplex* took sixty days to reach maturity.

b) Symptoms and yields. In all treatments *Atriplex* plants showed no visible symptoms of any nutritional disorders (Plate Id). Barley plants growing on both ash treatments remained stunted,

lacked tillering; the stems became purple, leaves were narrow and erect with yellow brown lesions on the margins and tips leading to leaf die-back. Later the leaves acquired dark brown spots which coalesced into streaks (Plate Ib). These symptoms are very similar to a combination of aluminium toxicity inducing phosphate deficiency together with manganese toxicity (Hewitt[5]). Spinach plants given both ash treatments also developed characteristic symptoms. Plants were dwarfed, leaf margins became scorched and finally dry and brittle. Yield data in the form of height, fresh and dry weights are summarized in Table VI. Whereas both ash treatments are

TABLE VI

Average weights of fresh and dry tops of plants grown on soil and ash

Treatment	*Atriplex*			Spinach			Barley		
	Height cm	Fresh wt., g	Dry wt., g	Height cm	Fresh wt., g	Dry wt., g	Height cm	Fresh wt., g	Dry wt., g
Soil	72·8	5·18	1·04	19·7	10·72	0.69	60·8	5·76	0·56
Ash + N.P.K.	62·8	9·98	1·55	14·2	7·69	0·54	32·6	0·45	0·089
Ash	10·2	0·59	0·04	5·8	2·25	0·14	29·9	0·28	0·057
Sig. Diff. $P = ·05$	6·31	1·67	0·457	4·14	2·53	0·234	6·14	1·37	0·059

detrimental to the growth of both barley and spinach, *Atriplex* flourishes best on the fertilised ash and the values for fresh and dry weight yields are greater on this treatment than on normal soil.

c) Nutrient status of plants

The fresh plant material was washed several times in distilled water before drying to remove aerial contamination (Jacobson[8]). All chemical analysis employed in this and subsequent experiments were made on the oven-dried material by the wet ash method of Wolf[17] with the exception that the digest was diluted with water and not Morgans reagent. The solution obtained by this method was used for the determination of the various elements mentioned. Nitrogen was determined by microkjeldahl.

The alkali and alkaline earth concentrations were measured with the flame photometer. The remaining ions were determined colorimetrically, magnesium by titan yellow (Lindner[9]); phosphorus by amino succinic acid (Wolf[17]); iron by thiocyanate (Treadwell and Hall[15]); manganese by periodate and phosphoric acid to avoid iron interference (Snell and Snell[14] and Nicholas[11]); aluminium by aurine tricarboxylic acid as modified by Chenery[1] and Nicholas[11].

The nutrition status of the three plants is summarized in Table VII *.

TABLE VII

Nutritional status of *Atriplex*, spinach and barley grown on soil and ash

Plant Material	Treatment	N	PO_4	K_2O	CaO	Mg	Fe	Mn	Al
Atriplex leaves	Soil	5·0	1·55	4·47	2·65	0·70	209	68	147
	Ash – N.P.K.	5·02	1·19	4·35	2·42	1·10	285	132	342
	Ash	4·02	1·21	3·51	2·85	1·06	285	130	349
Sig. Diff.	$P = ·05$	0·285	0·201	0·364	0·271	0·086	29·7	26.2	28·8
Spinach leaves	Soil	5·41	2·58	10·40	1·98	0·54	276	27	122
	Ash + N.P.K.	4·61	2·23	8·31	2·08	0·73	386	125	620
	Ash	3·98	2·18	6·72	2·15	0·62	393	111	630
Sig. Diff.	$P = ·05$	0·215	0·283	1·015	0·323	0·043	22.2	6·0	23·1
Barley tops	Soil	5·14	2·57	7·93	1·24	0·26	233	29	32
	Ash + N.P.K.	3·49	0·75	4·42	1·08	0·38	230	46	117
	Ash	3·11	0·85	5·00	1·05	0·38	212	52	105
Sig. Diff.	$P = ·05$	0·233	0·251	0·818	0·186	0·062	14·2	4·9	5·8

Some interesting points emerge from these analyses. *Atriplex* plants on ash alone contain significantly less nitrogen, phosphate and potash than the soil representatives. The addition to the ash medium of these elements raises their plant status somewhat but only nitrogen plant status reaches that of the soil control; the ash plants in both treatments have significantly greater Mg, Fe, Mn and Al status, in fact all ash plants with or without N.P.K. treatments accumulate more of these metals than the soil controls. The addition of nitrogen to the ash does not raise the status of this element in spinach and barley to that of the control. The phosphate levels of barley in both ash treatments is reduced to less than one third of the control. Each plant therefore responds differently to the ash metal content and nitrogen deficiency. *Atriplex* yields and some of its nutrients are lower but it shows no symptoms. Spinach is somewhat similar but it shows aluminium toxicity symptoms.

* In this and in subsequent tables the major elements are expressed as a percentage, the micronutrients as parts per million of the oven-dried material.

Barley is seriously affected through its phosphate content, which is again, in all probability, caused by ash aluminium.

2. *Crop indicator studies*

As the foregoing experiment showed that an excess uptake of manganese and aluminium had occurred in plants used these observations were extended to the use of a number of crop indicator plants which have been shown by Hewitt [4] to be sensitive to excess of these metals.

a) Culture procedure. The indicator plants used included two types of crop plants – *Brassicae*, unaffected by excess aluminium but sensitive to high manganese; a second group included dwarf bean var. masterpiece, runner bean, oats and tomato, since these plants quickly indicate excesses of aluminium. Runner bean indicates toxic quantities of both metals. Flax is stated to be insensitive to excess manganese whereas mustard is a manganese toxicity indicator (Hewitt) [4].

b) Symptoms. On 20th August 1954 seeds of all these plants were sown in replicate in two series of pots containing ash and soil respectively. The ash series was watered with a full culture solution (Hewitt) [6] and symptoms were noted as they occurred.

The brassicae developed marginal cupping and paling of leaf rims, the effect being most marked in cauliflower and least in swede (Plate IF).These symptoms closely resembled those induced by high manganese in sand culture (Hewitt [4]). Manganese toxicity symptoms developed somewhat late in mustard which initially grew more vigorously than the control but the leaves later developed scorched and brittle margins (Plate IC).

Tomato plants were slightly dwarfed (Plate IE); leaves and leaflets curled backwards; leaflets became dull purplish green in colour with brown dry tips recalling phosphate deficiency (Wallace [16]). Oats showed reduced tillering, plants were dwarfed with slightly chlorotic leaves, later showing die-back.

Dwarf beans produced squat plants, leaves became chlorotic and were shed prematurely. Runner beans showed a combination of excess manganese and aluminium symptoms, intervenal paling and necrotic spotting on margins (Mn) later leaf yellowing with premature defoliation (Al), (Plate IIA). Symptoms produced in tomato were very similar to those described by Hewitt [5] for excess

D E F

PLATE I. **A**: *Atriplex hastata* var. *deltoidea* forming an almost pure stand on newly tipped ash at Hams Hall. **B**: Barley ash symtoms. **C**: Mustard ash symptoms. **D**: *Atriplex* on ash (left) and soil respectively. **E**: Tomato leaves from soil (right) and ash plants respectively. **F**: Cauliflower ash symptoms.

aluminium. Irregularities in oats also correspond to symptoms induced by aluminium. The symptoms observed in dwarf beans are a combination of both aluminium and manganese toxicities (Hewitt[7]) Flax seedlings wilted, became yellow and ultimately died. These effects are probably due to aluminium. Hewitt[4] found no manganese toxicity symptoms for flax.

c) Plant nutrient status. Leaf samples of six of the original ten plants used were analysed for phosphate, aluminium and manganese. The remaining plants died or became defoliated. The leaf content of the three nutrients is presented in Table VIII in which it

TABLE VIII

Phosphate, aluminium and manganese content of indicator plants grown on soil and ash

Plant	Nutrient status					
	Phosphate		Aluminium		Manganese	
	Soil	Ash	Soil	Ash	Soil	Ash
Cabbage	1·119	1·051	23·2	64·1	18·7	42·7
Cauliflower	1·219	1·018	23·1	44.7	24·6	59·3
Mustard	1·802	1·800	18·0	45·0	30·8	80·0
Sprouts	1·227	1·075	15·5	56·8	15·5	68·6
Swede	1·442	0·983	30·4	52·3	35·7	76·5
Tomato	2·386	1·378	6·1	42·3	42·7	71·3

will be observed that all ash plants except mustard show a depressed phosphate uptake which is reduced to about half the phosphate status of the soil control in the case of tomato. It is noteworthy also that the ash tomato plants accumulate in their leaves some seven times the amount of aluminium absorbed by the soil controls.

3. *Nitrogen levels*

It has been observed in Table V that the ash is very deficient in nitrogen as far as normal crop plant requirements are concerned. The plant *Atriplex*, however, appears to tolerate this deficiency. Experiments were started to examine the behaviour of *Atriplex* under varying conditions of culture nitrogen using barley and spinach for comparative purposes.

a) Culture procedure. Plants were grown in water culture (Hewitt) [6], at five levels of nitrogen, 280 (control), 224, 168, 112 and 56 ppm. In March 1955 one week old seedlings of the three plants mounted on paraffined corks, three per cork, were placed in 1000-ml tall beakers darkened with black paper and connected to a root aerating system.

Mercury vapour lamps supplied auxillary lighting as required. Greenhouse temperature was maintained at 20°C ± 5°. The experiment ran for six weeks.

b) Symptoms and yields. Except that plants in lower levels of nitrogen were smaller *Atriplex* showed no symptoms of nitrogen deficiency (Plate IIᴅ). Spinach and barley both showed marginal scorching of the older leaves at lower levels of nitrogen. Table IX

TABLE IX

Fresh and dry weight yields at reduced nitrogen levels as percentage of control

N in culture, ppm	Tops		Roots	
	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
Atriplex				
224	107·22	106·22	91·28	107·70
168	78·48	88·56	68·52	75·06
112	50·92	59·58	44·52	44·16
56	41·82	51·84	39·83	21·68
Spinach				
224	78·12	80·00	56·82	63·08
168	21·88	24·12	27·40	31·81
112	15.88	19.77	16·91	21·31
56	8·11	15·56	8·78	13·26
Barley				
224	66·78	78·50	88·78	91·20
168	52·64	62·00	73·91	73·60
112	38·64	50·62	35·51	38·88
56	18·84	41·15	21·63	25·68

summarizes the fresh and dry weight yields of the respective plants. It will be observed that spinach is very sensitive to nitrogen, a reduction of the normal level by one fifth reduces the yield by twenty per cent. *Atriplex*, in contrast, shows an increase in fresh weigth at the same nitrogen level. The total nitrogen content of the three plants is affected in a manner very similar to the fresh weight response.

TABLE X

Leaf total nitrogen of plants grown at reduced nitrogen levels as percentage of the control			
N in culture, ppm	*Atriplex*	Spinach	Barley
224	103·20	93·48	87·50
168	94·38	89·00	78·20
112	74·72	62·82	65·42
56	74·72	55·38	46·76

c) Plant nutrient status. Table X summarizes the trend of total nitrogen in the three species as the applied nitrogen concentration is lowered. *Atriplex* is shown to be least affected by nitrogen shortage. Under similar conditions spinach showed a greater fall and its older leaves, in contrast to those of *Atriplex*, showed nitrogen starvation symptoms (Plate IIE). It seemed, therefore, that an analysis of upper and lower leaves of each species for nitrogen at the different levels might furnish information of the behaviour of nitrogen within the respective plants. These analyses were made at the end of the experiment and the results used to obtain Table XI. The

TABLE XI

Percentage ratio of nitrogen content of lower to upper leaves			
N applied, ppm		*Atriplex*	Spinach
280		56·5	58·8
224		58·8	56·2
168		67·2	54·8
112		68·0	53·7
56		68·9	39·2
Node number	upper	7 and 8	11 and 12
	lower	1 and 2	5 and 8

nodes were numbered from the base of the stem to the apex in each case. The data shows that at all levels of nitrogen applied the younger leaves have a higher nitrogen content. When the ratio of the lower (older) to upper (younger) leaves is calculated a rather striking result is obtained as shown in Table XI. This ratio increases as nitrogen is decreased in the case of *Atriplex* but the reverse is true of spinach. It seems, therefore, that the export of nitrogen from old to young leaves under nitrogen starvation is much slower in *Atriplex* than spinach.

4. Phosphate absorption

Experiments seemed to indicate that whereas *Atriplex* and spinach maintained a normal phosphate status on the ash, barley suffered phosphate deficiency even if additional soluble phosphate was added in excess. It was assumed that either ash aluminium or iron or both precipitated phosphate as insoluble aluminium or iron phosphates and that phosphate in this form is unacceptable to barley but may be taken in by *Atriplex* and spinach. A simple experiment was set up in April 1955 to ascertain if the phosphate status of plants is materially altered when its supply of phosphate is presented to it in an insoluble form.

a) Culture procedure. On 26th April, 1955 week-old seedlings of *Atriplex*, barley, tomato and two week old plants of cabbage and *Rumex acetosella* were transplanted to two series of water cultures, one containing its phosphate as potassium dihydrogen phosphate while insoluble calcium phosphate was used in a second series. *Rumex acetosella* was included because it is regarded as a plant exclusive to very acid soils where problems of manganese and aluminium toxicities exist, coupled with induced phosphate deficiency.

b) Symptoms. All plants grew normally. There was no evidence of phosphate deficiency.

c) Plant nutrient status. The experiment was terminated after four weeks and the plants were sampled, wet ashed and analysed. The nutrient status of tops of barley and the leaves of the remainder is summarized in Table XII. These data indicate that neither phosphate uptake nor that of other essential nutrients is

TABLE XII

Nutritional status of plants grown with different phosphate sources

Plant	PO_4	K_2O	N	CaO	Mg	Fe	Mn*	Al
With KH_2PO_4								
Atriplex	1·93	5·75	5·80	1·32	0·77	290	152	83
Barley	1·58	7·65	5·20	0·81	0·11	217	28	26
Tomato	1·28	4·38	4·60	2·17	0·45	267	66	60
Cabbage	0·98	6·30	4·60	1·71	0·28	157	26	46
Rumex	1·95	5·85	5·00	0·47	0.32	353	214	44
With $Ca_3(PO_4)_2$								
Atriplex	2·05	5·82	5·85	1·60	0·75	282	160	74
Barley	1·56	7·59	5·20	0·90	0·11	213	29	26
Tomato	1.30	4·05	4·75	2·18	0·44	215	71	28
Cabbage	1·01	6·12	4·80	1·92	0·27	150	26	44
Rumex	1·80	5·51	5·20	0·53	0.32	306	257	62

affected when an insoluble phosphate source is used. The only exception is a lower aluminium uptake from the calcium phosphate series in the case of tomato. Barley, along with other plants examined, can accept its phosphate from an insoluble salt.

5. *Ash/soil mixtures*

Previous observations have shown that the ash contains a sufficiency of all plant nutrients except nitrogen, in addition it may induce symptoms of aluminium and manganese toxicities in some plants. A series of trials were set up in which the ash was diluted by the addition of varying proportions of soil and a study was made of the effect of these mixtures as compared with either pure ash or soil.

a) Culture technique. The ash was mixed with garden soil in varying proportions by weight to give the following proportions of ash to soil: 12·5, 25, 50 per cent, using pure ash and pure soil as controls. (For mustard, cabbage, brussels sprouts and mangold). A more dilute mixture was prepared for barley at ascending levels of 2 per cent from 0 to 20 per cent ash. On 29th April 1955 the vegetable and barley seeds were sown in these mixtures in large pots. The pots were kept in the greenhouse and given tap water. Seedlings were thinned out to three per pot.

b) Symptoms

1. Mustard plants grew more vigorously on the ash soil mixtures than either soil or ash alone, but the plants showed typical manganese leaf scorch which increased as ash content became greater. These plants flowered earlier than the soil control (Plate IIF). Cabbage and sprouts (Plate IIB) showed very slight leaf cupping on all mixtures but did not otherwise differ in growth or vigour from the soil controls. Mangold ash soil plants were larger than soil representatives and were quite normal in appearance (Plate IIC). The sensitive barley showed typical ash symptoms when only 4 per cent ash was present.

2. Yield data and plant nutrient status.

On 24th June, 1955 the experiment ended. Barley tops and third and fourth leaves from other plants were sampled for fresh weight and chemical analysis. Fresh weight data are summarized in Figs. 1 and 2. Most plants show on increasing fresh weight response as the proportion of ash is raised until a maximum yield is reached followed

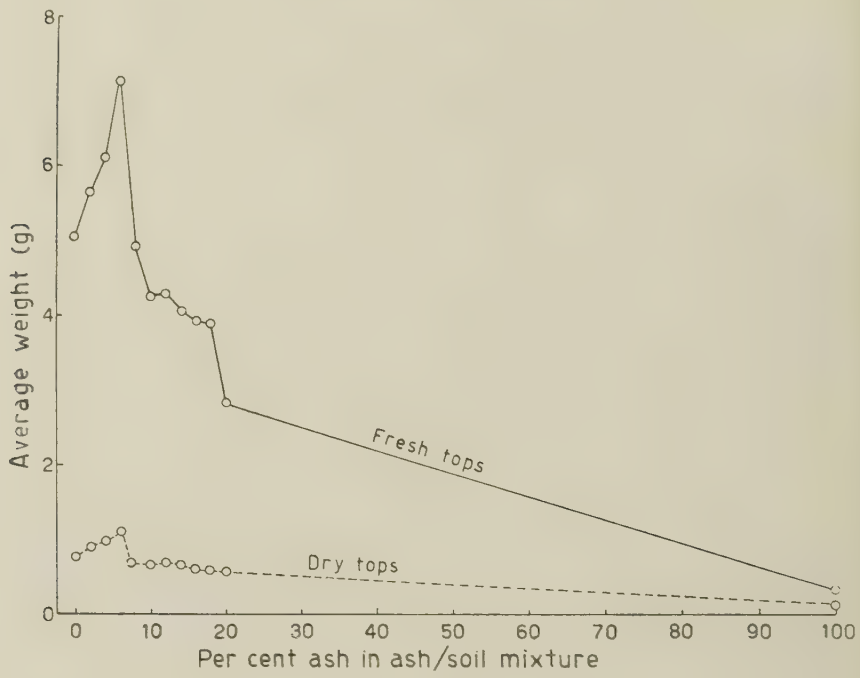

Fig. 1. Barley yields on ash/soil mixtures.

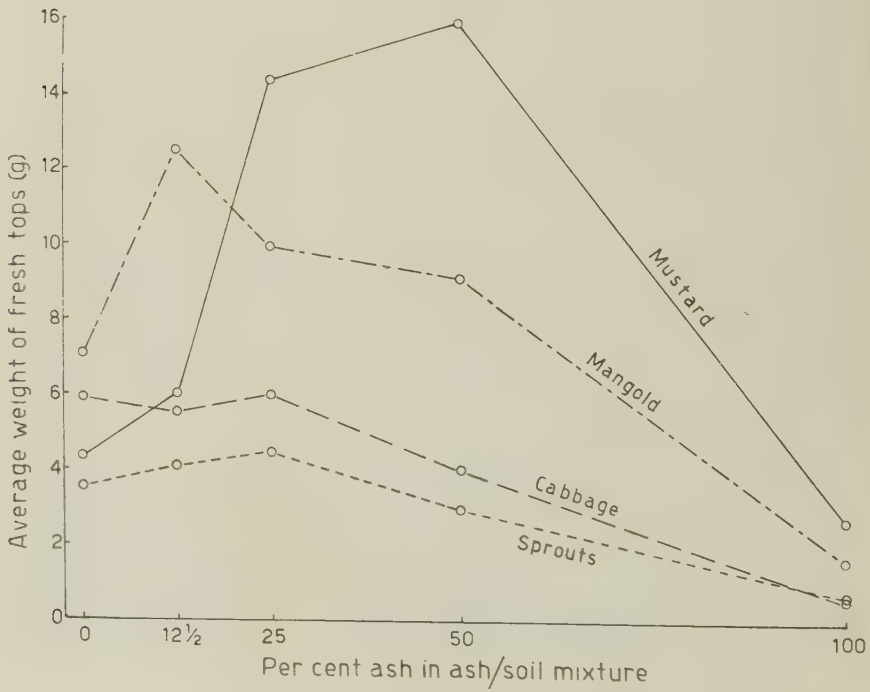

Fig. 2. Plant yields on ash/soil mixtures.

PLATE II. **A**: Runner bean ash symptoms **B**: Cabbage grown on different ash/soil mixtures (amount of ash used left to right: —0, 12½, 25, and 50 per cent respectively. **C**: Mangold; same mixtures as B. **D**: *Atriplex* grown in water culture at different N-levels; left 280, middle 112, and right 56 ppm respectively. **E**: Spinach grown in water culture at different N levels; same amounts as D. **F**: Mustard; same mixtures as B.

TABLE XIII

Concentration of plant nutrients in leaves of plants grown on ash/soil mixture

% Ash in ash/soil mixture	Mustard				Sprouts			
	N_2 %	PO_4 %	Mn ppm	Al ppm	N_2 %	PO_4 %	Mn ppm	Al ppm
0	3·90	1·825	30·8	10·0	4·30	1·925	10·0	10·0
12½	3·70	1·825	32·2	27·8	4·00	1·840	20·0	13·4
25	3·60	1·800	38·0	38·9	3·60	1·760	28·0	38·9
50	3·60	1·800	40·2	44·5	3·30	1·440	41·0	40·0
100	3·20	0·775	50·0	50·0	3·00	0·810	68·0	44·5
Sig. Diff. $P = ·05$	0·204	0·283	6·2	8·3	0·220	0·258	5.5	7.5
% Ash in ash/soil mixture	Cabbage				Mangold			
	N_2 %	PO_4 %	Mn ppm	Al ppm	N_2 %	PO_4 %	Mn ppm	Al ppm
0	4·30	1·830	16·0	20·0	4·20	2·035	14·2	29·0
12½	4·10	1·510	16·2	20·0	3·72	2·050	20·8	35·6
25	3·60	1·470	18·4	25·6	3·33	1·998	36·0	35·6
50	3·45	1·290	30·0	55·3	3·30	1·870	42·0	40·0
100	3·12	0·585	38·0	62·1	2·95	0·980	70·0	44·5
Sig. Diff. $P = ·05$	0·182	0·289	5·7	6·3	0·223	0·271	6.6	6.2

by a decline in response. The pure ash yields are the poorest in all cases. The ash soil ratios producing these maxima vary from as low as 6 per cent in barley to 50 per cent for mustard. Figures given in Table XIII for the nutritional status of some plants in the various mixtures show a fall in nitrogen but an increase in aluminium and manganese as ash increases. Phosphate status is not seriously reduced except in higher ash concentrations when fresh weight is falling.

IV. DISCUSSION

Exploratory enquiries into the plant nutritional potential of an important industrial waste indicate that it contains all the essential nutrients. It is deficient in nitrogen and also contains an abundance of metals which include essential plant micronutrients and a number of other non-essential metals such as nickel. It seems, however, that all these except manganese and aluminium are present in quantities which are harmless to plants, although concentrations of some of them *i.e.* Co, Cr, Fe, Ni, V and Zn do appear somewhat excessive in the acetic acid extract data of Table IV. There is no evidence in our experience of toxic symptoms produced by them. For example the

white necrotic streaks produced by toxic Ni on barley leaves are not caused by ash. Perhaps the high Fe status of the ash reduces any potential Ni effect as reported by Crooke *et al.* [2] in oats. Additional support to the hypothesis of the relatively harmless nature of many of the above mentioned metal ions in ash is furnished by data submitted in Table XIV, which summarizes the leaf metal status of cocksfoot plants grown by one of us on ash and soil respectively.

TABLE XIV

Leaf metal status of normal-healthy and ash-Dactylis plants

Metal	ppm oven dry material	
	Normal	Ash
Co	0·64	1·18
Cr	2·0	2·3
Cu	20·2	10·5
Fe	371	650
Ni	13·3	14·8
V	1·4	1·3
Zn	200·6	82

The data presented indicates that perhaps Co, Cr and Fe only might be suspected. However we obtained no evidence of either Co or Cr toxicities in our beet plants grown on ash. The available Fe status of the ash (Table IV) is admittedly quite high, whilst the amount of this metal is greater in the leaves of ash than of soil plants (Tables VII and XIV), barley being an exception in this respect.

The role of this metal in ash may require a separate more detailed investigation. The important conclusions drawn from observations so far clearly point to aluminium and manganese toxicity effects exhibited in different degrees on the various indicator plants employed. Three types of plant responses to excess aluminium may be distinguished: complete tolerance (*Atriplex*), partial tolerance with no phosphate problems (spinach) and great sensitivity to this metal (barley). Aluminium-induced root abnormalities were not evident with ash-barley, due no doubt to high ash calcium (Hewitt [4]).

Atriplex, barley and spinach show increased fresh and dry weight response when N, P and K are added to the ash but in *Atriplex* only does the ash N, P, K response exceed that of the soil. This result suggests that ash contains some property particularly favourable

to the growth of *Atriplex* which exerts a very powerful effect when adequate nitrogen is available to this plant.

Manganese toxicity symptoms clearly shown by brassicae grown on ash (Plate IF) confirm that abnormal quantities of manganese are available to plants.

The success of *Atriplex* under conditions of nitrogen shortage seems to be associated with its control of nitrogen drain from old to young leaves. It also suggests a degree of plasticity within this plant as far as nitrogen requirements are concerned since the genus *Atriplex* has often been considered somewhat in the nature of a nitrophilous plant.

The experiments conducted with mixtures of ash and soil indicate that there is a wide range of response by different species to varying proportions of ash to soil. The yield of the sensitive barley is effected by a much lower percentage of ash to soil than either mustard or mangold. Here again there is some indication of a beneficial nutritional effect of ash as far as crop plants are concerned. It is noteworthy also that, although mangold yields are reduced by a high percentage of ash the plants are entirely free from symptoms, in spite of the fact that the plants accumulate large quantities of aluminium and manganese in their leaves (Table XIII). Mangold behaves like the taxonomically related *Atriplex* in this respect.

It would appear probable that many other plants may tolerate the ash aluminium and manganese without much deleterious effect to their growth and yields. It seems that the nutrition problems of the ash may be compared with conditions prevalent within acid soils, podsols and the like which induce both aluminium and manganese toxicity symptoms in crop plants, except that the field pH of the ash is decidedly alkaline. Magistrad [10] has observed toxic aluminium concentrations in alkaline soils, and attributes the effect to a toxic soluble aluminate. It is quite possible that plants may obtain their aluminium in this form from the ash but confirmation of this point is needed.

The absence of the commoner weeds in the early natural succession on the ash may be related to its aluminium content. Gilbert and Pember [3] noted that toxic aluminium in acid soils do inhibit the growth of eleven common grassland weeds. *Atriplex*, however, is immune from this effect.

SUMMARY

1. Chemical analysis of fly ash reveals that it contains an abundance of metals in addition to normal plant requirements. However only manganese and aluminium appear to be available in quantities toxic to plants. The total nitrogen content of the ash is very low.

2. Barley and spinach grown on ash accumulate excessive quantities of aluminium and manganese in their leaves and exhibit symptoms of toxicities of these metals. *Atriplex hastata* var. *deltoidea* however which grows vigorously on the ash has a high aluminium and manganese leaf status but does not show toxicity symptoms.

3. The addition of a fertilizer to the ash increases the dry and fresh weight yields of all three plants but only in the case of *Atriplex* does the ash + N.P.K. yield exceed that of the soil control.

4. The ash aluminium induces phosphate deficiency symptoms in barley but does not affect the phosphate status of spinach and *Atriplex*.

5. A wide range of indicator plants were grown on ash and confirm both by symptoms and leaf analyses aluminium and manganese toxicities.

6. *Atriplex*, barley and spinach grown at reduced nitrogen levels gave lower yields than the normal control but symptoms of nitrogen deficiency which were evident in barley and spinach were not observed in *Atriplex*. The latter plant retains proportionately more nitrogen in its older leaves during nitrogen starvation than either barley or spinach.

7. Crop plants vary greatly in their tolerance to ash and soil mixed in different proportions. Barley is sensitive to low levels of ash showing symptoms and reduced yields whereas others, such as mangold, gave enhanced yields when quite a high proportion of ash was present.

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THE EFFECT OF FERROUS SULPHATE ON THE YIELD AND MANGANESE UPTAKE OF OATS ON SANDY SOIL FERTILIZED WITH PYROLUSITE

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A number of investigations during the present century have shown that the manganese nutrition of plants has been improved by fertilization with pyrolusite. Examples of increases in yield following fertilization with manganese dioxide, as assessed by pot and field experiments, have, in the case of wheat, been recorded by Giglioli[5], Salomone[16], Nazari[14], Ricci and Barbera[15] and, in the case of maize, by Sutherst and Ingle[21]. In some comprehensive studies, in which, amongst other things, the effects of increasing amounts of pyrolusite were compared with the effect of a number of more easily soluble manganese compounds given in increasing amounts to oats and horse beans in pot experiments, Leidreiter[12] showed that even if the sandy soil used was supplied with Thomas slag, which must be assumed to have contained manganese, the two crops still responded to added pyrolusite. A response to added pyrolusite was also found in mustard in a similar series of experiments made on a sandy, a loamy and a humus soil. Pot experiments with mangolds and sugar beet have also revealed a response to added pyrolusite as well as an increase in the sugar content of the beet; Fallada and Greisenegger[4] also found in pot experiments with sugar beet a response in root yield and sugar content to the addition of manganese dioxide. Furthermore, increases in yield resulting from the addition of pyrolusite have been demonstrated by Söderbaum[22] in pot experiments with oats and peas, by McHargue and Shedd[13] in sand culture experiments with oats, by Sherman and Harmer[17] in pot experiments with oats, and by

Iyer *et al.* [6 7] for the crops, barley, French beans, regi (*Eleucine coracana*), and tomato. Thus it seems established that it has been possible to apply manganese dioxide with a positive effect to a large number of plants under widely different climatic conditions and growing on many different soils.

It has been established by Leeper [11] that there is also a considerable residual effect of a manganese dioxide dressing since freshly precipitated manganese dioxide, uniformly mixed with a calcareous sandy loam from Mount Gambier, deficient in manganese, remained effective for eight years. Steenbjerg's [18] investigations into the influence of various reducing agents on the contents of available manganese in the soil also included a pot experiment with oats on a sandy soil deficient in manganese, in which it was shown that the manganese effect of a precipitated manganese dioxide was doubled when sodium sulphite was also added to the soil.

Jones and Leeper [9 10] in a study on the manganese effect of various synthetic manganese oxides and natural ores, applied to oats on soil deficient in manganese, have shown by pot experiments that pyrolusite prepared in the dry way had no effect, whereas pyrolusite prepared in the wet way was very effective as a manganese fertilizer. Electron microscopy showed that the latter had a large specific surface consisting of very small particles with low crystallinity, whereas the former was dense and visibly crystalline.

Previous investigations (Boken [1 2]) have shown that a ferrous sulphate dressing could act as a manganese dressing on several soils and for several crops, presumably as a result of its ability to reduce higher manganese oxides in the soil; in laboratory experiments more manganese was extracted with a solution of magnesium nitrate to which ferrous sulphate was added than with a solution of magnesium nitrate alone. In the investigations reported below it has been tested whether plants were able to extract more manganese from a sandy soil dressed with precipitated pyrolusite when ferrous sulphate had been added to it than from a soil dressed with pyrolusite to which no ferrous sulphate had been added. At the same time attempts have been made to elucidate the influence of the age of the precipitated pyrolusite on its manganese effect.

METHODS

The *pot experiments* were carried out in cylindrical zinc pots coated on the inside with paraffin wax. The pots were fitted with external pipes for watering from the bottom of the container; broken flints were used as drainage material. The capacity of the pots was 20 l, and their surface area, 500 sq. cm.

Measurement of pH was made with the glass electrode.

The *calcium carbonate* and *carbon contents* of the soils were determined by using the Scheibler and the Ter Meulen apparatus respectively.

The manganese content of crops was determined colorimetrically as permanganate after wet ashing with a mixture of sulphuric- and perchloric acids. Divalent manganese was oxidized in the neutralized solution by potassium persulphate in the presence of sulphuric acid and silver ions.

The *total manganese* content of the soil was determined by refluxing 20 g of air-dried soil with 200 ml 20 per cent hydrochloric acid for one hour, and the content of manganese then determined as above.

The exchangeable manganese of the soil was estimated by shaking for one hour, 20 g soil with 200 ml *M* magnesium nitrate. *The content of reducible manganese in the soil* was determined by shaking another 20 g soil with 200 ml *M* magnesium nitrate containing 10 mg hydroquinone. The difference between the amounts of manganese found by these two methods is denoted as reducible manganese.

The two samples of pyrolusite used in the experiments were finely ground up in an agate mortar. By means of a JEL set of sieves it was arranged that, as far as possible, the particle size of the two samples was the same, the fraction of diameter 100–200 μ being used in each case.

The amount of reducible manganese in the pyrolusite was determined by shaking 1 g pyrolusite for an hour with 200 ml *M* magnesium nitrate with a pH of 7 and containing 25 mg hydroquinone. *Exchangeable manganese* was estimated by shaking for one hour 10 g pyrolusite with 100 ml *M* magnesium nitrate.

The *X-ray analyses* were made with chrome radiation and a vanadium filter in 19-cm Bradley cameras. For the *X-ray diffractometer* investigations an apparatus of the type XRD-3, General Electric X-ray Corporation, U.S.A., was used.

EXPERIMENTAL RESULTS

The pot experiments described below were made with a sandy soil, poor in nutrients, taken from a field of the State Experiment Station at Store Jyndevad. According to Jensen and Kjær [8], the clay fraction (0-2 μ) constituted 3 per cent, 8.4 per cent of which was quartz and the silt fraction (2–20 μ) 2 per cent, 40 per cent of

which was quartz; only 17 per cent of the soil particles passed through a 20-μ sieve.

The soil was limed to a pH of about 6 immediately before the experiment was begun. The carbon content of the soil was 1.99 per cent C; the soil contained 1.4 ppm exchangeable manganese, 1.8 ppm reducible manganese and 60 ppm total manganese. According to Danish conditions it was therefore a soil with a very low content of total and reducible manganese and with an exchangeable manganese content of such an order of magnitude as to indicate a slight deficiency in this nutrient.

As a basal dressing for the experiment the following was used per pot: 3 g N as NH_4NO_3, 4 g K as KCl, 4 g P ás H_3PO_4, 3 g Mg as $MgSO_4.7H_2O$, 0.25 g Cu as $CuSO_4.5H_2O$, and 0.011 g B as $Na_2B_4O_7.10H_2O$. All salts used were Merck's A.R. and were added in solution.

The experimental fertilizers were $MnSO_4.4H_2O$ and $FeSO_4.7H_2O$ both Merck A.R., and two samples of technical pyrolusite, both prepared in the wet way of Merck, but at different times. One was imported into this country at the end of 1938 and was probably prepared in 1938, the other was made by Merck in February 1955. In what follows the samples are termed "Pyrolusite 1938" and "Pyrolusite 1955", respectively. The experimental fertilizers were uniformly mixed with the upper 10 kg soil in the pots.

The surface area of the pyrolusite samples was determined by the B.E.T. method (Brunauer, Emmett and Teller [3]), evaporation being carried out at 100° C for 16–18 hours *; 13.6 sq. m g was found for the "Pyrolusite 1938" and 13.8 sq.m g for the "Pyrolusite 1955".

X-ray diffractometrical examinations of the two samples of pyrolusite made in Januari 1956 showed that the crystalline phase in both samples consisted of very pure β-manganese dioxide.

The content of total manganese was determined at 57.0 per cent in "Pyrolusite 1938" and at 56.8 per cent in "Pyrolusite 1955".

According to the data of Table I Column 1, 0.125 g water soluble Mn as $MnSO_4$ was compared with 0.125 g reducible Mn as pyrolusite. It would have been more expedient to have added a larger amout

* I wish to offer my best thanks to Mr. S. T. Jakobsen, M.Sc., who made these measurements in this department, and to Mr. E. Jensen, M.Sc., who made the X-ray diffractometrical examinations in the Chemical Laboratory of the Royal Veterinary and Agricultural College.

of reducible manganese as pyrolusite, since in this way we might have expected greater and surer responses; unfortunately only a very limited amount of "Pyrolusite 1938" was available.

By analysis, "Pyrolusite 1938" was found to contain 1500 ppm and "Pyrolusite 1955" 1600 ppm reducible manganese, hence 83 and 78 g per pot of "Pyrolusite 1938" and "Pyrolusite 1955", respectively, were added in the pot experiments.

No water-soluble and no exchangeable manganese were found in the two samples of pyrolusite.

50 g ferrous sulphate was added per pot; calculated on the basis of the total amount of soil in the pot, this corresponds to the 10 mg hydroquinone per 20 g soil which was used for the determination of the content of reducible manganese in the soil.

The test crop was oats *var.* Victory; the pots were kept in a glass-house during rainy weather and the soil was maintained at a moisture content equal to 40 per cent of its total water holding capacity calculated on an air-dry basis.

Notes on plant growth

The oats were sown on April 18th and germinated uniformly, germination being completed on May 4th. On May 27th the plants were thinned to 25 plants per pot. In the first week of June slight symptoms of manganese deficiency were observed in the oats in all pots, these symptoms having disappeared completely about July 1st. During July the plants were sprayed repeatedly with 1‰ nicotine solution to control aphids. The oats began earing on July 8th and had finished earing on July 11th. The oats were harvested when ripe from August 11th to August 15th.

As a dressing with manganese gives the most distinct response in grain yield, straw production has been omitted from the harvesting data presented in Table I.

As appears from the table, yield increased with increasing additions of manganese as manganese sulphate. The yield and amount of manganese absorbed per pot were of the same magnitude for both "Pyrolusite 1938" and "Pyrolusite 1955". The yield for 0.125 g reducible manganese as pyrolusite is slightly smaller than that for 0.125 g water-soluble manganese as manganese sulphate; the opposite is the case as regards the total amount of manganese absorbed per pot.

TABLE I

Treatment	g dry matter per pot		ppm Mn in dry matter	mg Mn per pot	
	Yield	Increase in yield		Absorbed	Increase in absorption
Control	22.2	—	125	2.8	—
0.125 g Mn as $MnSO_4$	30.3	8.1 *	100	3.0	0.2
0,250 g Mn as $MnSO_4$	37.0	14.8 **	120	4.4	1.6
0.125 g reducible Mn as „Pyrolusite 1938"	28.1	5.9	130	3.7	0.9
0.125 g reducible Mn in „Pyrolusite 1955"	27.2	5.0	135	3.7	0.9
50 g $FeSO_4$	22.9	0.7	120	2.7	—0.1
50 g $FeSO_4$ + 0.125 g Mn as $MnSO_4$	29.6	7.4	120	3.6	0.8
50 g $FeSO_4$ + 0.125 g reducible Mn in „Pyrolusite 1938"	38.8	16.6 **	300	11.6	8.8
50 g $FeSO_4$ + 0.125 g reducible Mn in „Pyrolusite 1955"	47.9	25.7 **	250	12.0	9.2
S.E. ±	2.7	3.9			

* 0.05 level ** 0.01 level

Addition of ferrous sulphate alone did not raise the yield of grain or the amount of manganese absorbed per pot by the grain. When ferrous sulphate was combined with 0.125 g manganese as manganese sulphate, the yield and the total uptake of manganese were found to be of the same magnitude, as when 0.125 g manganese as manganese sulphate was added alone.

Dressing of the soil with both ferrous sulphate and pyrolusite produced a considerably higher yield and total uptake of manganese per pot than when the soil was dressed with pyrolusite alone. The response was about 50 per cent higher in the case of "Pyrolusite 1955" than in the case of "Pyrolusite 1938". On the other hand there was no difference between these two cases in the amounts of manganese absorbed per pot.

DISCUSSION AND CONCLUSIONS

The methods of analysis used for the determination of the content of exchangeable manganese (1.4 ppm) and reducible manganese (1.8 ppm) in the soil were apparently successful in characterising a sandy soil containing only small amounts of manganese available to the plants. The fact that the soil was deficient in manganese is borne out by the significant increasing response to increasing amounts of

manganese added, just as the relative content of manganese in the grain first decreased, then increased with increasing additions of manganese sulphate (Steenbjerg [19]). A content of 1.4 ppm exchangeable manganese in the soil did not indicate a soil highly deficient in manganese. Indeed, this was corroborated by the harvesting results, since the yield of the "control" was comparatively high, viz. 22.2 g grain.

The content of reducible manganese in the soil, 1.8 ppm, was extremely low, and since similar yields of grain were obtained from the "control" and from the soil dressed with ferrous sulphate, just as the total manganese uptake per pot was of equal order, it must be assumed that the content of active manganese oxides in the soil was so low that it could not obscure the fertilizing effect of the added pyrolusite.

The response to 0.125 g reducible manganese added as "Pyrolusite 1938" and "Pyrolusite 1955", respectively, was of the same magnitude, but slightly lower than the response to 0.125 g water-soluble manganese added as manganese sulphate. The difference however, is not significant. It is possible that the amount of manganese extracted from the pyrolusite in the laboratory, is larger than that which could be extracted by the plants from the pyrolusite in the soil under the conditions of reduction-oxidation prevailing in the pot experiments. Perhaps a less rigorous treatment of the pyrolusite in the laboratory, *e.g.* by using a smaller amount of hydroquinone, would have given better agreement between the analytical method and the ability of the oats to extract manganese from the pyrolusite added to the soil.

Though the fertilizing effect of manganese sulphate was apparently slightly greater than that of pyrolusite, the total amount of manganese taken up per pot was somewhat lower. This need not imply any discrepancy since the fertilizing effect may be dependent on adequate amounts of manganese being available for the plants during particular period, which, in the case of manganese, is known to be in the very early summer. Since the soil in the pots was kept at a constantly low water capacity, it is probable that the reduction of pyrolusite proceeded slowly, but perhaps over the whole growing season in such a way that manganese may have been available to the plants during the whole of this period, but to an insufficient degree at the beginning of the season. Hence the yield from pyro-

lusite may be comparatively low but the total absorption of manganese comparatively high compared with manganese sulphate, which is presumably most easily available to the plants at the beginning of the growing season.

It would appear from the above, that, in investigations into the effect of sparingly soluble fertilizers, it is important that the absorption of the nutrient in question by the plants is also investigated as a function of time.

Addition of ferrous sulphate to the soils dressed with 0.125 g reducible manganese per pot as pyrolusite gave a response which, in the case of "Pyrolusite 1938", was twice as high, and in the case of "Pyrolusite 1955" was three times as high as the response to addition of 0.125 g manganese as manganese sulphate. In the case of both pyrolusite fertilizers the total amount of manganese absorbed per pot was four times as high as that with the dressing of manganese sulphate. These results support the assumption previously advanced (Boken[2]) that the manganese effect of ferrous sulphate is due to its ability to reduce higher manganese oxides in the soil.

By extraction of "Pyrolusite 1938" and "Pyrolusite 1955" with hydroquinone a content of reducible manganese was found in the two fertilizers of 1500 ppm and 1600 ppm respectively. As the B.E.T. measurements showed that the two samples of pyrolusite had the same surface area, viz. 13.6 and 13.8 sq.m g, respectively, the observed difference between the contents of reducible manganese in the two samples may indicate a different crystal structure, which could not be established by the X-ray diffractometrical examination, the amount of manganese available to the plants thus being also different. The results of the pot experiments reveal, to a much greater extent than the extraction analyses with hydroquinone, that there is a difference in the contents of manganese available to the plants between the two samples of pyrolusite when the soil is also dressed with ferrous sulphate, the response to "Pyrolusite 1955" being about 50 per cent higher than that to "Pyrolusite 1938". Since the same amount of reducible manganese and the same number of manganese atoms on the surface in the two pyrolusite fertilizers are involved (Steenbjerg[20]), it is probable that the observed difference between the effects of the fertilizers in the presence of ferrous sulphate is due to a difference in the crystallinity of the fertilizers. No difference between the two pyrolusite fertilizers was observed in the absence of ferrous

sulphate. This may be due to the fact that such small amounts of manganese were added that the responses to these became relative small and uncertain.

SUMMARY

In pot experiments with oats on a sandy soil deficient in manganese and with an extremely low content of reducible manganese, the effect of 0.125 g reducible manganese in two pyrolusite fertilizers (β-manganese dioxides) made in 1938 and 1955, respectively, was compared with the effect of 0.125 g water-soluble manganese as manganese sulphate.

On soil to which no ferrous sulphate was added, the response to manganese added as "Pyrolusite 1938" and "Pyrolusite 1955" was of the same magnitude, but slightly less than the response to manganese added as manganese sulphate. The difference between the effect of pyrolusite and that of manganese sulphate was not, however, statistically significant.

On soil to which ferrous sulphate had been added, a response was obtained to "Pyrolusite 1938" twice as high, and to "Pyrolusite 1955" three times as high as the response to manganese sulphate. These results support the suggestion [2] that the manganese effect of ferrous sulphate is due to its ability to reduce higher manganese oxides in the soil.

Since soil dressed with ferrous sulphate gave a response to added "Pyrolusite 1955" which was about 50 per cent higher than the response to "Pyrolusite 1938", and since, furthermore, the same amount of reducible manganese and the same number of manganese atoms on the surface of the two fertilizers were involved, it is probable that the difference between the effects of the two pyrolusite fertilizers found under these circumstances is due to difference in their crystallinity.

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SOME OBSERVATIONS ON THE MICROBIAL ACTIVITY IN REMOISTENED AIR-DRIED SOILS *

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INTRODUCTION

For many years investigators concerned with the evolution of carbon dioxyde or the uptake of oxygen by remoistened air-dried soils have noted an initial period in which gas exchange far exceeded that observed for fresh but little disturbed samples of the same soils [2 10 11 13 14 19 20].

The stimulatory effects of air drying are generally explained on the basis of physical and chemical changes resulting in an increase in readily available nutrients for the soil microflora [1 6 10 18]. In this respect the experimental work of Lebedjantzev [7] is noteworthy. This investigator has shown that the process of air drying brings about the following changes: (*a*) small changes in the solubility of mineral substances, (*b*) large increases in the solubility of organic substances, (*c*) large increases in nitrogen and phosphorus, (*d*) large increases in NH_4-N and amide nitrogen, and (*e*) a sharp decrease in numbers of micro-organisms.

It is generally felt that the resulting burst in respiratory activity immediately following remoistening is due to the utilization of these readily available nutrients by the soil flora. Several objections have been raised to this theory on the basis of the marked decrease in bacterial numbers in air-dried soils and the frequent observation that maximum numbers of micro-organisms generally occur much later than the observed high point in respiratory acitivity [13 16 17]. Goodding and McCalla [3] have advanced a possible explanation

* Contribution No. 411.

for the behaviour of the microflora on air drying and rewetting of soils. These authors suggest that the process of air drying converts the microbial forms into inactive states such as spores or other forms not actively respiring. When conditions are again made favourable for growth by the addition of moisture, the organisms pass into a vegetative state or into a highly active form.

The present paper deals with an investigation of the early phases of respiration of remoistened air-dried soils in an attempt to clarify this period of adjustment.

MATERIALS AND METHODS

Soils

The four soils used in the following studies are referred to as Soils X, FC, BC and NG. The term 'fresh soil' used in the text refers to soil samples refrigerated in a moist condition until used. Air-dried soils refer to samples air dried at room temperature for 48 hours prior to use.

Manometric techniques

To determine oxygen uptake 4.0 g samples of the 1–2 mm fraction of air-dried and fresh soils were placed in Warburg vessels. Additions of water were made directly to the soils to bring them to 60% of their water holding capacity (W.H.C.). Due allowance was made for differences in the initial moisture content of the soils as well as for differences in their water holding capacities. Carbon dioxide was removed from the atmosphere in the vessels by the addition of 0.2 ml of 20% KOH to the centre well. Unless otherwise stated vessels were allowed to equilibrate for one half hour prior to closing the manometers and commencing the readings. All studies were undertaken at 30°C and results are expressed as rates in terms of μl/g air-dried soil/hour. Daily rates of oxygen uptake were calculated on average pressure changes over a period of 6 hours and are expressed as μl/g/hour.

Carbon dioxide evolution was determined by the conventional Direct Method [15].

Bacterial counts

Bacterial numbers were determined by plate counts on soil extract agar after 14 days' incubation at 26 C. Numbers are expressed per gram of oven-dried soil. In order to determine numbers of bacteria at different intervals for comparison with respiratory data, 25-ml flasks, containing 4.0 g of soil at 60% W.H.C. were held in a water bath at 30°C. At appropriate intervals the contents of the flasks were removed and plated.

Preparation and concentration of soil extracts

Soil extracts were prepared by adding 50 ml of distilled water to 50 g of

soil, allowing to stand for one hour and shaking vigorously for 10 minutes. The resulting suspensions were then clarified by passage through a Buchner filter, and the filtrates made up to 50 ml with distilled water.

Aqueous extracts were concentrated by freeze drying and resuspended in known volumes of 80% ethanol for chromatography and in 10% n-isopropyl alcohol for amino nitrogen determinations.

Chromatography

Known amounts of the ethanol solutions of the concentrated aqueous extracts were spotted on Whatman No. 4 filter paper for ascending chromatography with butanol-acetic acid-water as solvent [9]. After 18 hours the papers were removed, dried, sprayed with ninhydrin (0.2% in 95% ethanol) and developed at 45°C.

Amino nitrogen determinations

Amino nitrogen was determined by the Chloramine-T method [5] in a conventional Warburg apparatus.

RESULTS

Soil respiration studies

Respiration data of air-dried and fresh samples of four soils over a period of 7 days are given in Figure 1. Particular attention is drawn to the higher initial respiratory activity of the air-dried soils over

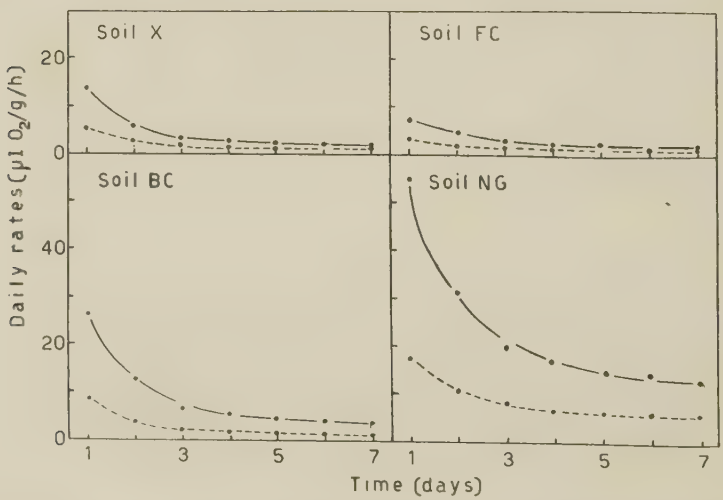

Fig. 1. Daily rates of oxygen uptake of fresh and air-dried soils after remoistening. Air dried ——; Fresh – – – –.

the corresponding fresh samples. This 'initial burst' is typical of that previously noted by various investigators [10 14 20]. After the first day, respiratory rates of the air-dried soils decrease and gradually approach the respiratory levels displayed by the fresh soils. In seven days the respiratory activity of the air-dried samples of Soils X and FC are approaching the levels of the fresh samples, whereas the activity is still somewhat higher in the dried samples of the considerably more active Soils BC and NG. Other workers have shown that the time necessary for respiratory activity of remoistened air-dried soils to decrease to a fairly constant level may vary from as little as 1–2 hours to 1–2 weeks depending on the soil [1 2].

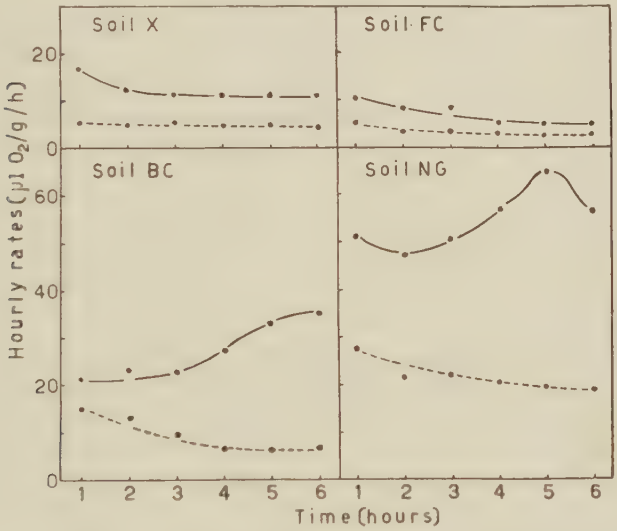

Fig. 2. Hourly rates of oxygen uptake of fresh and air-dried soils after remoistening. Air dried ——; Fresh – – – –.

Hourly respiration rates of air-dried and fresh soils following remoistening are given in Figure 2. These data well illustrate the higher respiratory activity of the air-dried samples of all soils. Some fluctuations are noted in the respiration of the two more active air-dried soils (BC and NG) with the respiratory peak occurring between five and six hours. The less active air-dried samples (X and FC) demonstrate highest oxygen uptake in the first hour.

Bacterial numbers

Bacterial numbers were determined for both fresh and air-dried samples of all soils and these data are given in Table I. In all cases air-drying has reduced the numbers of viable organisms.

TABLE I

Soil	Numbers of bacteria in soil	
	Fresh	Air dried
X	160×10^6	116×10^6
FC	100×10^6	85×10^6
BC	44×10^7	12×10^7
NG	65×10^7	17×10^7

In view of the lower numbers in the air-dried soils and the higher respiratory activity which they demonstrate, a series of plate counts were made on fresh and air-dried samples of Soils X and NG. After remoistening these soils the numbers were determined at 0, 1, 2, 4, 6, 9, and 12 hour intervals during the first day followed by five daily counts.

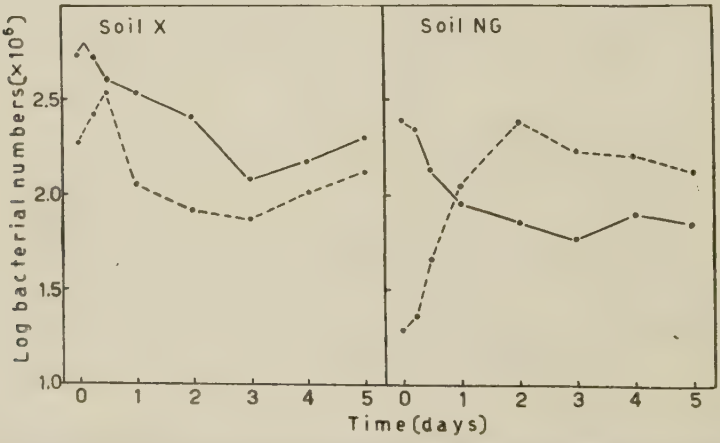

Fig. 3. Bacterial numbers in fresh and air-dried soils for a period of five days after remoistening. Air dried – – – –; Fresh ——.

Comparative numbers of bacteria for fresh and air-dried samples of the two soils are illustrated in Figure 3. Bacterial numbers in fresh Soil X show a slight initial increase followed by a gradual decrease to the third day. In the comparable air-dried sample there

is a fairly rapid increase in numbers during the first half day after which numbers follow more or less the same pattern as the fresh soil except at a lower level. In the more highly active NG soil, numbers of bacteria in the fresh sample rapidly decline during the first few days while a rapid increase in numbers is observed in the air-dried soil in the same period. During this phase of multiplication the numbers in the air-dried sample exceed those of the fresh soil and remain higher over the 5-day period.

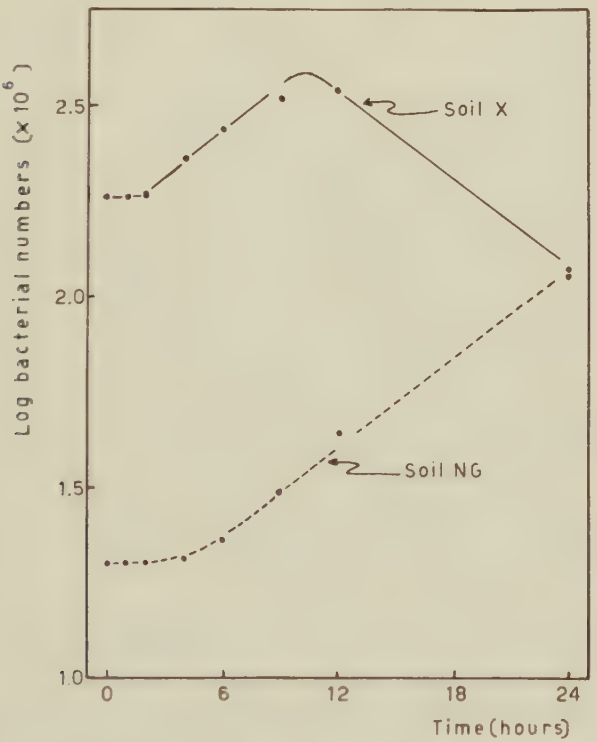

Fig. 4. Bacterial numbers in air-dried soils during the first twenty-four hour after remoistening.

Bacterial numbers of the air-dried samples of Soils X and NG are compared over a period of 24 hours in Figure 4. Both curves illustrate a three-hour lag period followed by a phase of logarithmic increase. In Soil X this logarithmic phase lasts for a period of 9 hours and in Soil NG this phase extends into the second day (Fig. 3).

The high respiratory activity of the air-dried samples immediately following remoistening is surprising in view of the lag period where numbers of organisms remain constant for a three hour period. The fact that numbers of bacteria in the corresponding fresh samples are greater (Fig. 3) though with considerably lower respiratory activity (Fig. 2) suggests that during the observed lag phase the bacteria in the air-dried soils are more active. It would appear that, immediately on remoistening, the bacteria remaining after drying, pass into a phase of physiological youth which is characterized by extremely active metabolism and a rapid increase in mass but not in numbers [2].

Following the initial lag phase and characterized by a rapid rate of cell multiplication there is a period of logarithmic increase. During the first 6-hour period following remoistening of the soils there are some variations in the metabolic rates, particularly in the more active soils (Fig. 2). The maximum respiration of Soil NG observed at 5 hours actually occurs very early in this phase of increase, at a time when there may still be a number of organisms which are changing from the very active phase of physiological youth to the less active logarithmic phase.

It is also possible that the concentration of available substrates will influence respiratory activity during the early hours. Inasmuch as these studies were carried out with unsupplemented soils, it is possible that the process of air drying will have altered the available nutrients in the soils to varying degrees with a resulting influence on respiratory levels and metabolic rates.

The presence of free amino acids and ninhydrin-positive substances in air-dried and fresh soils

Aqueous extracts of air-dried and fresh soils were prepared and concentrated to determine the presence of free amino acids or peptide materials in the soils. A chromatogram of these extracts is presented in Figure 5. In all soils, free amino acids were detected by these methods, with the amounts of these substances showing a marked increase through air drying. No attempt has been made at this point to identify the individual amino acids extracted but the results of quantitative determinations of total amino-N [5] are given in Table II.

Although the Chloramine-T method failed to detect the small

TABLE II

Liberation of free amino acids by fresh and air-dried soils

Soils	Amino-N * (μl CO_2)	
	Fresh soil	Air-dried soil
X	—	40.2
FC	—	60.2
BC	—	139.0
NG	—	521.0

* In leachate equivalent to 50 g soil, after subtraction of reagent blanks.

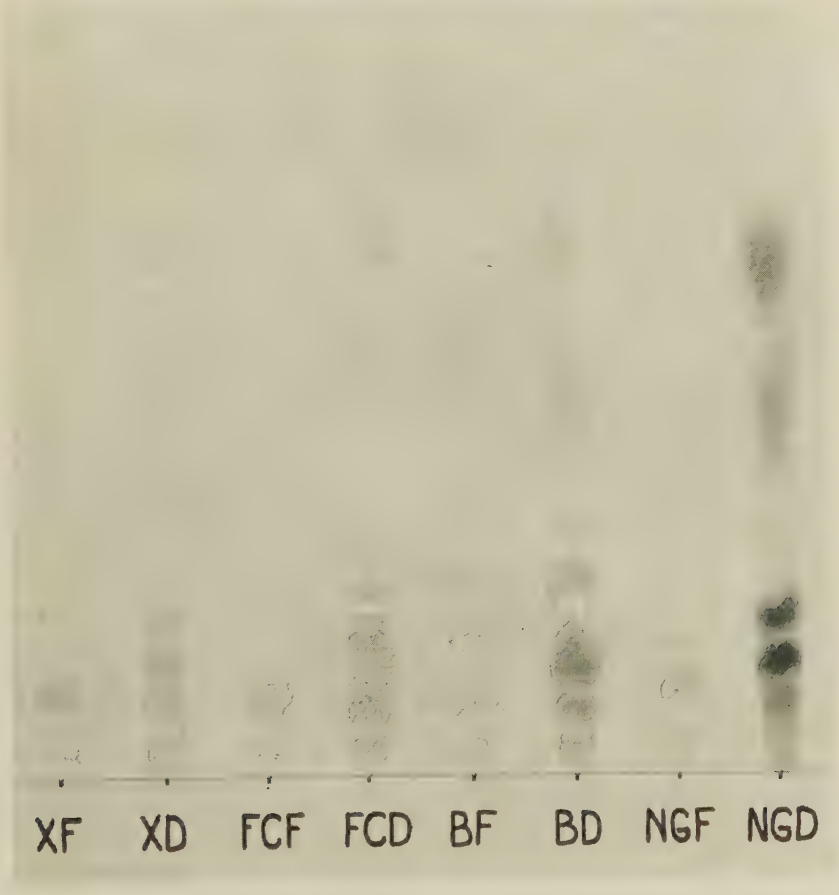

Fig. 5. Paper chromatogram of ninhydrin-positive substances in the leachates of fresh and air-dried soils. Extracts of 5.0 g of soil spotted in each case. Fresh soils suffixed by F and air-dried soils by D.

amounts of amino compounds observed chromatographically in the fresh soils, the quantities in the air-dried soils are of interest. A definite parallel exists between the amounts of nitrogenous material released on air drying with the increased metabolic activity of the various air-dried soils observed in Figure 2.

The organic matter, total-N determinations and C : N ratios of the soils are presented in Table III.

TABLE III

Chemical nature of soils				
Soils	O.M. *	Total-N †	% C	C : N
X	11.44	0.41	6.64	16.24
FC	5.25	0.32	3.05	9.41
BC	7.14	0.35	4.14	11.90
NG	20.74	1.02	12.03	11.77

* Determined by wet combustion method.
† Kjeldahl method.

The C : N ratios are of particular interest in that, with the exception of Soil X, the ratios approach 10 : 1 which is generally considered normal for temperate soils. Under these conditions a release of available nitrogen in proportion to the O.M. content of the soils is to be expected. In Soil X where a wide C : N ratio is observed, conditions are different with the result that a much smaller proportion of available nitrogen will be released [12]. Such being the case, the expected and observed concentrations of amino acids and other nitrogenous compounds released by the four soils parallel the increased metabolic activity on air drying.

The effects of soil sterilization on gas exchange

A number of investigators have suggested that the high initial respiratory activity of air-dried soils is due in part to the chemical and physical action taking place during the air drying process. It is felt that during this period gases are adsorbed which are later released on remoistening, resulting in the anomalous respiratory values observed during the early hours.

In order to determine non-biological gas exchange, air-dried samples of Soil NG were sterilized by treatment with formalin and by autoclaving. The sterile soils were then placed in Warburg vessels and their activity determined in terms of oxygen uptake and carbon

dioxide evolution. Immediate gas exchange of these soils was determined by equilibrating the vessels prior to remoistening. Readings were taken immediately following this addition of water.

In the formalin treated soil no gas exchange was noted during the 6-hour period investigated. Evolution of carbon dioxide and uptake of oxygen in the autoclaved soil are given in Table IV along with the respiratory data for air-dried and fresh samples.

TABLE IV

Gas exchange of sterile, air-dried and fresh samples of Soil NG immediately after the addition of water

Time in minutes	Autoclaved		Air dried		Fresh	
	O_2	CO_2	O_2	CO_2	O_2	CO_2
10	+21.0	+ 9.0	— 9.1	+ 7.8	— 4.0	+ 3.3
20	+18.9	+ 9.0	— 20.6	+ 18.1	— 7.5	+ 6.4
30	+16.8	+ 9.4	— 32.5	+ 30.6	— 11.7	+ 9.8
60	+ 8.6	+10.6	— 55.2	+ 50.9	— 23.5	+19.5
120	— 1.0	+12.3	—102.8	+ 91.3	— 44.4	+36.8
180	— 9.4	+13.9	—152.8	+119.5	— 61.5	+51.3
240	—16.9	+16.1	—210.2	+167.1	— 78.9	+66.9
300	—23.1	+18.3	—275.3	+219.5	— 93.3	+79.1
360	—29.3	+18.3	—332.3	+260.8	—107.9	+91.9

* Accumulated O_2 and CO_2, μl/g of soil.

It will be noted that during the first ten-minute interval the autoclaved soil evolved a total of 21.0 μl of oxygen and 90 μl of carbon dioxide. Further investigation indicated that this initial evolution actually occurred during the first minute following the addition of moisture to the soil. No immediate bursts of these gases were observed on remoistening the non-sterile fresh and air-dried samples. Although some oxygen is absorbed and some carbon dioxide is evolved by the sterile soil during the six-hour period, the total gas exchange occurring in this soil is quite insignificant as compared with that occurring in the air-dried and fresh soils. It is felt that any evolution or uptake of gases resulting from chemical action taking place during air drying would be far less than that observed after heat sterilization This would suggest that non-biological reactions do not contribute significantly to activity of remoistened air-dried soil.

DISCUSSION

In this investigation an attempt has been made to clarify existing theories concerning the increased metabolic activity in air-dried soils

immediately following remoistening. All of the soils studied have demonstrated much higher respiratory rates in air-dried samples as compared with fresh soils despite a lower bacterial population after air drying. Quantitative studies of the bacterial populations of two soils under conditions paralleling those of the respiratory studies have evidenced two distinct growth phases: an initial three-hour lag period followed by a phase in which the organisms multiply logarithmically. During the initial lag phase which immediately follows remoistening, the metabolic activity in the air-dried soils is high and it would appear that the organisms present are in a state of physiological youth. From this phase they pass to a phase of logarithmic increase in which the numbers of bacteria increase proportionally with a decrease in metabolic activity. This results in only a slight change in overall respiratory activity.

The length of the phase of logarithmic increase varies in turn with the soil used. Examination of extracts of air-dried and fresh soils for free amino acids has given evidence of a much higher concentration of these substances in the air-dried soils. The concentration of amino acids is low in all fresh samples but the amounts released through air drying increase proportionally with the organic matter contents of the soils providing the C : N ratios are not changing. Since the level of metabolic activity of air-dried soils over fresh soils varies directly with the amounts of nitrogenous materials released on air drying, it is felt that the presence of readily available substrates is responsible in part for the increased respiration noted in air-dried soils on remoistening.

Some attention has been given to the possibility that the high initial activity observed in air-dried soils is due to the release of adsorbed gases produced by chemical action during the air drying process. Some evolution of oxygen and carbon dioxide was noted from a heat-sterilized soil immediately on remoistening, but the amounts produced were slight. Assuming that the chemical action taking place on heat sterilization is far in excess of that occurring during air drying it is felt that the large differences between the respiratory activity of air-dried and fresh soils are almost completely due to biological activity and not to chemical action.

One important aspect of the problem of air drying which has been neglected is the probable qualitative shift in the microbial balance during the process. The fact that there is a marked decrease in

numbers has often been noted but no comparative studies have been recorded concerning the morphological, nutritional, or biochemical differences of the bacterial flora before and after air drying. It is quite possible that on air drying the soil microflora is selectively decreased leaving a population whose nutritional requirements are satisfied by the nutrients (amino acids, *etc.*) produced by the process. On remoistening, these organisms, having an adequate supply of suitable substrate, pass into a highly active state. In preliminary investigations carried out in this laboratory, no significant differences were noted in the nutritional classification of some 200 isolates of air-dried and fresh soils[8]. Some differences were noted in the biochemical activities of these isolates, with a much higher percentage of ammonifying organisms being present among the isolates from the air-dried soil. Further investigations are being undertaken along these lines and will be reported in a later communication.

SUMMARY

Comparative studies on the respiration of four air-dried and fresh soils show that in all cases a higher level of metabolic activity is attained in the air-dried soils following remoistening. The degree by which metabolic activity increases in remoistened air-dried soils varies directly with the concentrations of free amino acids and other nitrogenous materials released by the air drying process. No appreciable gas exchange was noted through chemical action alone and it is felt that the increased respiration of air-dried samples over fresh soils is due mainly to biological activity.

Quantitative studies of the bacterial flora of air-dried soils indicate a short lag period followed by a phase of logarithmic increase during the early stages following remoistening. These results are discussed in terms of the bacterial population existing after air drying and its relationship to the high respiratory activity observed.

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VOL VIII

1. p.98, caption to Fig. 1, and also p.99, caption to Fig. 2, "mg vitamin B_1" should read "μg vitamin B_1".
2. p. 102, Fig. 3A, the numbers of the curves from top to bottom "1, 2, 4, 2, 5" should read "1, 3, 4, 2, 5".
3. p. 102, caption to Fig. 3, "0.25 mg Vitamin B_1" should read "50 μg vitamin B_1".
4. p. 114, line 5, "from grassland on river clay soils in the central region of the country" should read "from grassland on marine clay soils".
5. p. 114, caption to Fig. 10, "interactions" should read "influences".
6. p. 114, caption to Fig. 11, "interactions with" should read "influences of".
7. p. 119, caption to Fig. 19, "interactions" should read "influences".
8. p. 179, line 9, "90 μl" should read "9.0 μl".